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PLANT AND SOIL

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PLANT AND SOIL

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A STUDY OF THE AVAILABLE PHOSPHORUS STATUS OF SOILS BY PHOSPHORUS-DEFICIENT TOMATO SEEDLINGS

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Certain plants manifest characteristic phosphorus deficiency symptoms when grown in media low in available phosphorus and the symptoms disappear when supply of available P is restored. Little effort has been made to utilize this fact to assess quantitatively the phosphorus fertility status of soils.

Anthocyanin pigments are known to be formed in many plants when the supply of phosphorus is reduced; they disappear as adequate amounts of phosphorus are present in the soil. The biochemical steps leading to the formation of anthocyanin in phosphorus deficient plants are not clearly understood [7] [8], but it is known that anthocyanin formation results from metabolic changes in the cell. In the present investigation the change in concentration of anthocyanin pigments during recovery from phosphorus starvation was taken as a measure to predict the phosphorus status of soils. In addition to the measurements of anthocyanin concentration, analyses for chlorophyll and total phosphorus were also undertaken.

TECHNIQUE

The tomato plant (*Lycopersicum esculentum* Mill.) which shows characteristic phosphorus deficiency symptoms and can be easily grown in the greenhouse was selected as indicator plant. Two early varieties were used in the present study and no difference due to variety was observed.

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The seeds were germinated in sand using distilled water to supply moisture. After emergence of the cotyledonary leaves, a 1 : 1 dilute Hoagland nutrient solution devoid of phosphorus was used to irrigate the medium (KH_2PO_4 was replaced by KCl). After proliferation of the first leaves, minus-phosphorus Hoagland solution of normal concentration was used to supply elements other than phosphorus.

Depending upon the season of year, the time required for the appearance of phosphorus deficiency symptoms varied considerably. Therefore, by selecting seedlings of the same stage of development or physiological age for transplanting to soils, the data from several experiments could be compared. The first symptoms of phosphorus deficiency were the appearance of purple (anthocyanin) colour in the leaves and stems plus a marked retardation in growth rate. It was followed by deepening of the anthocyanin colour and arresting of the growth processes. As the phosphorus deficiency progressed the leaves appeared pale green or yellowish-green, and the pair of cotyledonary leaves turned yellow and dropped off. In the present study, this physiological period has been termed the *critical stage of phosphorus deficiency*, at which time the tomato seedlings were transplanted to soils. It took from 3 to 7 weeks for the appearance of the symptoms associated with the critical phosphorus deficiency.

Soils used in the present study were collected from the plow layer of various field experiments conducted by the Agronomy Department [3]). The soil samples were dried and mixed, screened through a 4-mm sieve, and stored until ready for use.

Glazed pots containing 800 grams of the mineral soils or 500 grams of the organic soils were used for growing the test plants. Nutrients other than phosphorus were added to all soils at the rates indicated in Table I. Four levels of phosphorus were added — 0, 75, 150, and

TABLE I

Amount of nutrients added to soils		
Nutrient	Chemicals used	Pounds per acre *)
N	NH_4NO_3	200
Mg	$MgSO_4.7H_2O$	23
S	$MgSO_4.7H_2O$	30
K	KCl and/or KH_2PO_4	165

*) Based on 2,000,000 lbs. of soil.

300 pounds P_2O_5 per acre; each treatment was replicated four times. KH_2PO_4 was used to supply the required amount of phosphorus to the soils. KCl was added wherever necessary to make the potassium level the same in all treatments.

All experiments were conducted in the greenhouse. During winter months the daylight was supplemented in the evening by light from tungsten filament lights thereby making the total light period about 13 hours. The soil moisture was kept at approximately field capacity. The plants were allowed to grow for a period of 25 $\pm$ 3 days after transplanting to soils.

To obtain a representative sample for the determination of anthocyanin and chlorophyll pigments, a cork borer, diameter 0.55 cm, was used to cut 160 discs of leaf laminae from the plants in each treatment. After uniform mixing this sample was divided into two equal parts for anthocyanin and chlorophyll analysis. The remaining plant material was dried at 70°C for 24 hours.

A modification of the procedure of Thimann and Edmundson [7]) was used for the determination of anthocyanin pigments. The discs were steeped in 10 ml of a 0.1 *N* HCl solution. Preliminary experiments showed that a steeping period of 24 hours, was sufficient to extract the anthocyanin pigments. After the 24-hours steeping period, the whole mass was mixed in a Waring blendor using a small cup. The contents were mixed for 2 minutes and filtered through Whatman # 42 filter paper. The optical density of the filtrate was measured in a Coleman Jr. Spectrophotometer, model 6. 525 mμ wavelength, the region of minimum transmission, was elected for measuring the optical density of the HCl-anthocyanin extract.

For chlorophyll determination, the discs were steeped in 10 ml of 95 percent ethanol for 24 hours. Before mixing with a Waring blendor, an additional 25 ml of ethanol was added. After a 2-minute mixing period, the whole mass was filtered through Whatman # 42 filter paper. Optical density of the filtrate was determined in the Coleman Jr. Spectrophotometer at a wavelength of 660 mμ, the region of minimum transmission [1]). The data have been presented as an anthocyanin or chlorophyll index and are expressed as optical density $\times$ 100 per 80 leaf discs, which had a total area equal to 37.87 cm^2 on both sides of the leaf punches.

The plant material was dry ashed according to the procedure used

by McAuliffe [4]), and total phosphorus was determined by the molybdivanadophosphoric acid method [2]).

RESULTS AND DISCUSSION

In the present investigation 16 soils were studied in six different experiments. Selected data from these experiments will be discussed to show the utility of the method.

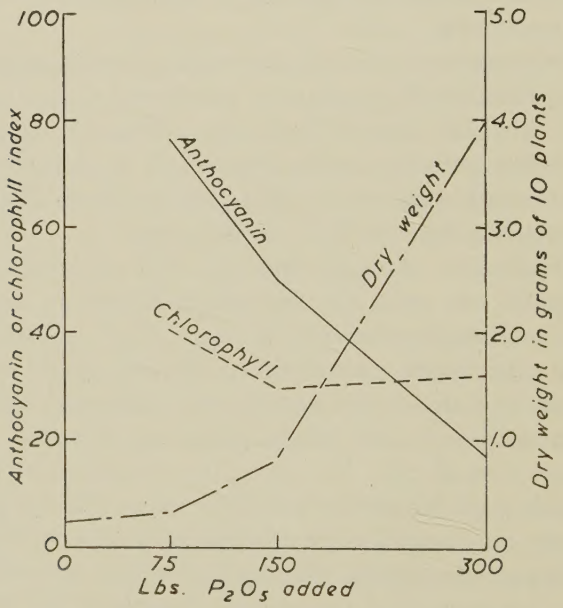

Fig. 1. Dry weight and chemical composition of tomato plants grown on Lima silt loam.

As shown in Figure 1 the Lima silt loam soil gave a significant response to added levels of phosphorus. The anthocyanin index dropped rapidly as the level of added phosphorus was increased. In 8 of the 16 soils studied this same general trend was observed. This group of soils has given significant response to phosphorus in the field under many situations. Thus, in the case of these 8 soils, there was an inverse relationship between the added phosphorus and the anthocyanin index of the plant leaf material.

The second group of soils were those in which little or no response to added phosphorus was observed. As shown in Figure 2 the previ-

ously heavily fertilized organic soil fell in this category as did 4 other soils used in these experiments. The anthocyanin index was very low even with no phosphorus added, indicating a much higher level of available phosphorus in these soils than in those discussed previously. The increase in yield, however, still was rather significant, though much smaller than in the soils of the first mentoined groups; but it must be remembered that the transplanted tomato seedlings were highly deficient in phosphorus so that undoubtedly their requirement for phosphorus was higher than that of a normal plant.

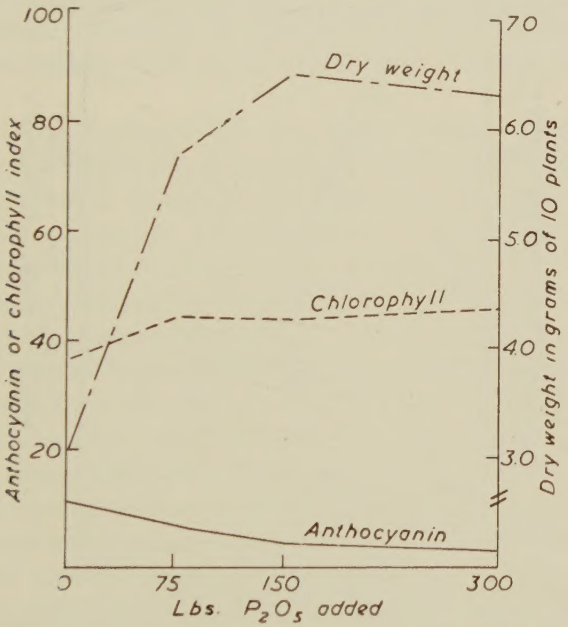

Fig. 2. Dry weight and chemical composition of tomato plants grown on previously heavily fertilised organic soil.

With the acid Mardin silt loam which was very high in soluble iron and aluminium and low in phosphorus as determined by the methods of P e e c h and E n g l i s h [5]), the response to phosphorus was much less (Figure 3) than where the iron and aluminium content was not a factor. At the high rates of application of phosphorus the anthocyanin index decreased appreciably, but with little relative effect upon yield. Therefore, in this instance the low response may have been due to the retardation of growth caused by the presence

of iron and aluminium in toxic concentrations and might not have been due to the lack of available phosphorus. The slight increase in yield at higher levels of added phosphorus might have been due to a decrease in the high levels of iron and aluminium resulting from the greater application of phosphorus.

From the results presented above it seems that a high anthocyanin concentration in the leaves signified a low amount of available phosphorus was present in the soil and a low anthocyanin index signified a high concentration of available phosphorus. The anthocyanin index was inversely related to the available phosphorus

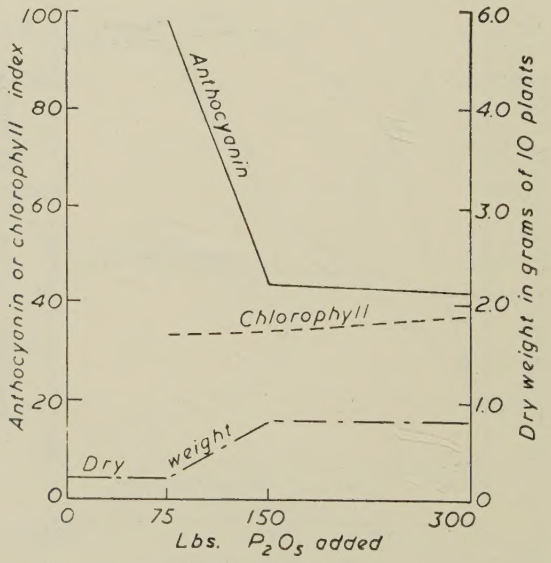

Fig. 3. Dry weight and chemical composition of tomato plants grown on Mardin silt loam.

status of the soil, but when other factors were retarding plant growth, the anthocyanin concentration was not necessarily related to dry matter production.

Usually there was an increase in the phosphorus content of the plants with an increase in the phosphorus concentration of the medium (Table II). While there was no general correlation between the phosphorus content of the plant and dry matter production, there appeared to be, however, a critical range in the phosphorus content of the shoot. Below this critical range an increase in the

TABLE II

Phosphorus content of tomato plants, expressed as percent on dry weight basis				
Soil type	Level of added phosphorus			
	0	75	150	300
Lima	—	0.08	0.18	0.21
Organic soil	0.38	0.42	0.50	0.57
Mardin	—	0.12	0.18	0.18

phosphorus content was associated with increased growth but above this range an increase in the phosphorus content of the plants had little influence upon yield. In these experiments the critical level of phosphorus in the plant was normally between 0.15 and 0.20 per cent.

At the levels of phosphorus used in these experiments there was very little effect upon the chlorophyll concentration of the leaves. It must be concluded, therefore, that there is no relationship between the phosphorus content and chlorophyll formation in the tomato plant, except where the deficiency is very severe.

Those soils evaluated in these experiments which have responded to applied phosphorus under field conditions have given growth and anthocyanin index curves similar to those shown in Figure 1 for the Lima soil. The increase in dry matter production over the range from 0 to 300 pounds of added P_2O_5 has been at least tenfold. For those soils evaluated by this technique which have not responded to phosphorus under field conditions the growth and anthocyanin index curves have been similar to those in Figure 2. The increase in dry matter production for these soils over the range from 0 to 300 pounds of added P_2O_5 has always been less than fivefold.

The experiment using the Mardin silt loam may have been an exception since the yield response to added phosphorus was low. The anthocyanin index and the phosphorus content indicated, however, that there was an increase in availability of phosphorus at the higher levels. High levels of heavy metals present in this soil, therefore, could very likely have been the cause of poor growth as has been shown by Schmehl, Peech and Bradfield [5]). This technique may, therefore, prove useful to deliniate heavy metal interference and phosphorus deficiency in plants.

SUMMARY

Several important New York soils were evaluated for their phosphorus fertility status in the greenhouse by growing tomato plants and determining the relationship between the anthocyanin content of the plants and their response to added phosphorus. In general, there was an inverse relationship between the anthocyanin content of the leaves and increases in dry weight production when phosphorus only was the limiting factor.

It appears that the method presented may have considerable value as a greenhouse technique to evaluate the phosphorus fertility of soils.

The problem of evaluating the relationship between the anthocyanin content and phosphorus response when heavy metals interfere remains to be solved. Nevertheless, this technique may be valuable to segregate heavy metal interference from phosphorus deficiency.

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A COMPARISON OF MANGANESE SOURCES USING TOMATO PLANTS GROWN ON MARL, PEAT AND SAND SOILS

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The availability and fate of manganese contained in a soil, returned in plant residues or added as fertilizer, are governed by soil release and retention mechanics. Such manganese relationships are recognized as both complicated and divergent in their properties from one soil type to another (Mulder and Gerretsen[7]), Leeper[6]), Heintze and Mann[2]), Timonin[12]), Hemstock and Low[3])). From these reports soils alkaline or neutral in their reaction, particularly if highly organic, usually are involved where manganese-deficiency in the crop has been identified. In Florida the unfertilized marl soils and alkaline Everglades muck are commonly considered to be deficient in soil manganese in a form available for crops. The need for manganese from manganous sulfate for the marl and Everglades soils was recognized about 1930 by Schreiner and Dawson[9]) and Skinner and Ruprecht[10]). Since that time it is common practice to use up to 50 pounds per acre of manganous sulfate annually in the mixed fertilizer applied to these soils and the earlier use of manure which supplied sufficient manganese if applied at heavy rates is now not an economical practice. The present study was made to evaluate manganous oxide, gamma manganese dioxide ore, and a chelated manganese source at comparative rates with manganous sulfate on these soil types. Tomato plants were used as the indicator crop and division of the analysis of plant parts was made so that a basis for tissue analysis might be possible from these studies.

EXPERIMENTAL

Two soils were chosen that are known to produce crops suffering from

severe manganese deficiency unless sufficient manganese is included in the mixed fertilizer. Perrine marl, pH 8.3, was obtained from a virgin area. This soil is a silt loam composed chiefly of calcium carbonate with varying amounts of coarse and well-decomposed organic matter. Everglades peaty muck, pH 7.0, was taken from an area where admixing of underlying marl with the soil had occurred near a drainage ditch and this was 88 per cent organic matter. The third soil type, Scranton sand, pH 5.0, was removed from the plow-depth layer of a field that had grown crops many years without manganese fertilization and this soil contained 5 per cent organic matter, 2 per cent silt, about 2 per cent clay and the remainder sand. Each soil was sieved while moist and transferred into glazed pots. For each soil type a weight of soil was chosen to fill the pots about one inch from the top and pots in each randomized experiment received similar weight of soil. Each soil was fertilized at rates deemed necessary to have no limiting element except manganese. For Perrine marl the applications as pounds per acre were as follows: superphosphate (20% P_2O_5) 1000, ammonium nitrate 400, sulfate of potash 350, sulfate of magnesium 400, ferrous ammonium sulfate 15, copper sulfate 20, zinc sulfate 20, borax 1, ammonium molybdate 0.1, and the manganese sources at 0, 10, 40, 80, 120, expressed as equivalent pounds MnO. For the Everglades peaty muck the fertilizer rates were: superphosphate 400 and 2000, ammonium nitrate 165, sulfate of potash 350, sulfate of magnesium 350, ferrous ammonium sulfate 15, copper sulfate 50, zinc sulfate 20, borax 1, molybdate 0, and the manganese sources 0, 10, 20, 40, 80, 160 pounds of equivalent MnO per acre depending on the source. The Scranton sand was split into two series of pots, one of which was limed using hydrated lime to a $CaCO_3$ equivalent of six tons giving a final pH of 7.5, and the other pots left unlimed. The limed and unlimed soil received superphosphate at two levels, 300 and 1500 pounds superphosphate (20% P_2O_5), ammonium nitrate 300, sulfate of potash 350, sulfate of magnesium 400, ferrous ammonium sulfate 15, copper sulfate 10, zinc sulfate 10, borax 1, ammonium molybdate 0.1, and the manganese sources 0, 10, 40, 80, 120 pounds equivalent to MnO per acre. In all the experiments the superphosphate and manganese sources were mixed together and applied as solids, one half of which was banded on the soil surface exposed after removal of the top 5 inches of the soil and the other half mixed with the top 5 inches of the soil. The other fertilizer ingredients were applied as a solution with the ammonium nitrate, sulfate of potash and sulfate of magnesium being applied one half before planting. The pots were planted with Rutgers variety tomato plants, from a commercial seed-bed, and the precaution was taken to wash the roots well with de-ionized water prior to planting. After the first 30 days of growth the remainder of the potash, nitrogen and magnesium salts were applied in equal increments every 10 days. The plants on the Everglades peaty muck received a small amount of the ammonium nitrate initially and equal increments after 30 days when it was observed that nitrate production in the soil was insufficient to maintain adequate nitrogen for the plants. The effect of phosphate on

the uptake of manganese from the Everglades peaty muck and Scranton sand was studied as a sub-experiment within the randomization.

Description of manganese sources

The gamma manganese dioxide is a sample of the pulverized ore mined in Katanga, Africa. This ore is of a type useful in battery manufacture. X-ray diffraction gives major spacings in angstroms 2.36, 2.15, 2.05; plateau 2.41–2.45; plateau 4.00–4.03; a doublet 3.13 and 1.98. The supply is reported to be extensive and the analysis consistent, usually 56% Mn, 1% Fe, 0.02% Cu, 0.03% Ni, 0.03% Co and Pb-free. Mechanical analysis of the sample used in this experiment gave the particle sizes as per cent to be fine sand 26, silt 50, coarse clay 4.0, colloidal clay 20. By the hydroquinone and normal calcium nitrate extractions of Jones and Leeper[1]) three such successive leachings of 0.100 g of this oxide gave 4600, 3900 and 470 micrograms of easily reducible manganese. After thorough mixing, the amount of the oxide required per pot was weighed on the basis of the manganese content and the rates calculated to pounds MnO per acre on an area-basis of the pot.

The manganous oxide source was a sample of the above ore which had been electrically reduced by the Manganese Reduction Company, Baltimore. X-ray diffraction indicated manganosite or MnO was formed and the major spacings were 2.24, 2.58 and minor at 2.05, 3.45, 3.37, 3.13. The sample analyzed 68 per cent Mn. Mechanical analysis showed particle size distribution of 18% fine sand, 61% silt, 4.6% coarse clay and 16% colloidal material. By similar leachings to that indicated above, the easily reducible manganese in 0.10 g samples was found to be 4050, 3800 and 1020 micrograms Mn. Weights of the dry sample were taken again on the basis of the manganese content and as equivalent pounds MnO supplied per acre.

The chelated manganese source was manganous ethylene-diamine-tetra-acetate, hereafter termed MnEDTA. It was prepared by the stoichiometric reaction of chemically pure manganous carbonate and EDTA. The dry product contained 15.8% Mn and was ground to pass a 140-mesh sieve. Hydroquinone and subsequent calcium nitrate treatment as for the oxides resulted in complete solution of the MnEDTA. Rates per pot were obtained using the Mn-content of the dry product and were expressed as the pounds of MnO supplied per acre.

The manganous sulfate is chemically pure $MnSO_4 . 1H_2O$, 32.5% Mn and screened to pass 140-mesh sieve. Weighings were made on the same basis as the other sources.

Harvesting procedure

The plants were harvested when tomato fruit commenced ripening. The fruits were weighed, washed in water containing a detergent, rinsed with de-ionized water and stored in a refrigerator prior to wet digestion.The stem and leaves were dried in paper bags to constant weight at 70°C.

Chemical methods

The fruit per pot was placed in a beaker and evaporated to low volume. Wet digestion was made using 100 ml of nitric acid, 10 ml of perchloric acid, taking each sample to strong fumes of perchloric acid. Further small additions of nitric acid and then of 30% hydrogen peroxide were made to insure complete digestion. Total amount of reagents per sample was recorded to permit correction for Mn and Fe in the reagents and reagent blanks were carried through each set of samples being digested. The stem and leaves were ground in a Wiley Mill. The samples were dried again and 15 grams taken for the wet digestion which was conducted in wide mouth Erlenmeyer flasks with Pyrex cover glasses. Reagents and procedure were essentially the same as for the fruit. The wet ash was transferred to a volumetric flask and made to volume with redistilled water. A 10-ml portion was taken for iron analysis by the *O*-phenalthroline-citrate method described by Sandell[8]). After centrifuging, the transmittancy was measured through a 5-cm light path at 525 μ for the manganese determination in a Cenco Photelometer and through a 1-cm path at 510 μ in a Beckman B Spectrophotometer for the iron determination.

RESULTS

Visual symptoms

Symptoms of manganese deficiency appeared about one month after planting on leaves of plants growing on Perrine marl receiving no manganese and the lowest rate of the γMnO_2 source and also after about three weeks on leaves of the tomato plants growing on the Everglades peaty muck not receiving manganese, on the lowest rates of MnO_2 source and at all rates for the MnEDTA. The chlorosis, necrotic lesions and small rolled leaves observed in this experiment were similar to the description provided by Schreiner and Dawson[9]) and Wallace[11]). For less severe deficiency the chlorosis was scarcely evident but necrotic lesions were present. In some cases, particularly the γMnO_2 source on Everglades peat, the older leaves only showed the characteristic minute lesions and the new leaves were normal in appearance, and the authors classify such plants as slightly manganese deficient. In presenting the data the leaf symptoms are described as very manganese deficient (V), manganese deficient (D), slightly manganese deficient (S), or normal (N). Other deficiency was not observed for the leaves or fruit on the Perrine marl and this applied also for those growing on the Everglades peaty muck except for a few fruit with blossom-end rot and a marginal burn on plants receiving the MnEDTA.

Physiological leaf symptoms of deficiency or toxicity from manganese or iron were not observed for leaves on plants growing in the experiments using Scranton sand. Indications of magnesium deficiency were corrected by subsequent applications of sulfate of magnesium. The unlimed Scranton soil produced tomato fruit with a high incidence of blossom-end rot but this did not correlate with the manganese or phosphate treatments. The fruit from tomatoes grown on the limed Scranton were without blossom-end rot. Comparison of the manganese sources on the basis of manganese deficiency, dry weights and chemical analysis is presented in the tables. Statistical analyses were made to assist in the comparisons.

Data from tomatoes grown on Perrine marl

The yield data from the outdoors pot study using tomato plants grown in Perrine marl are shown in Table I. Using the least signifi-

TABLE I

Comparison of leaf symptoms, dry weight of leaves and stem (P) and fresh weight of fruit (F) by rates and sources of manganese applied in pots with Perrine marl. Average weight and least significant differences are given in grams

Rate as MnO per acre		Manganese sources			
		MnO	γMnO_2	$MnSO_4$	MnEDTA
10 pounds	P	57.0 N	51.3 D	53.4 N	46.3 D
	F	387	293	496	402
20 pounds	P	—	—	—	46.3 D
	F	—	—	—	491
40 pounds	P	55.8 N	60.8 S	55.4 N	—
	F	441	381	488	—
80 pounds	P	68.3 N	69.2 N	61.7 N	54.9 S
	F	401	472	421	585
120 pounds	P	69.8 N	59.7 N	48.5 N	50.6 N
	F	368	437	614	553
L.S.D. 0.01	P	20.2	No manganese applied		
	F	137	P 43.7 V		
L.S.D. 0.05	P	15.0	F 231		
	F	101			

cant difference (L.S.D.) at the 5 per cent and 1 per cent probability levels between treatments, the yields were affected by the rate and source of manganese applied. Weight of stem and leaves was greater than the check plants where the manganous oxide was applied at the two highest rates; at all but the lowest rate of the

γMnO_2 source; and at the second highest rate of the $MnSO_4$ source. Fresh weight of fruit at the time of harvest was greater than that found for the very Mn-deficient check plants for all rates and sources except the lowest rate of the γMnO_2 source. Part of the variation in the leaves and stem weights is attributable to the fruit yields since a plant with larger fruit weight was generally smaller than those bearing less fruit. This may explain the stem and leaves from the plants treated with $MnSO_4$ and MnEDTA weighing less than for the oxide sources.

TABLE II

Comparison of total manganese intake found in leaves and stem (P) and in the fruit (F) by rates and sources of manganese applied in pots with Perrine marl. Average intake and L.S.D. values in micrograms Mn

Rate as MnO per acre		Manganese sources			
		MnO	γMnO_2	$MnSO_4$	MnEDTA
10 pounds	P	499 N	477 D	1227 N	275 D
	F	169	42	157	53
20 pounds	P	—	—	—	377 D
	F	—	—	—	45
40 pounds	P	897 N	665 S	1290 N	—
	F	240	54	232	—
80 pounds	P	1359 N	1217 N	1036 N	908 S
	F	207	155	205	73
120 pounds	P	1850 N	812 N	2490 N	1715 N
	F	190	174	294	382
L.S.D. 0.01	P	612	No manganese applied		
	F	80.6	P 209 V		
L.S.D. 0.05	P	451	F 33		
	F	59.5			

Comparison of the manganese intake is shown in Table II. There was a highly significant increase in the manganese of the leaves and stem at the three highest rates of the MnO source, the two highest of the γMnO_2 and MnEDTA, and at all rates of the $MnSO_4$. The Mn-deficiency symptoms are in accord with this measure of intake of manganese except for the MnO at the lowest rate. Plants showing the early necrosis at the second rate of the γMnO_2 and third rate of the MnEDTA gave significant increase in manganese intake over the check. The manganese found in the fruit was highly significantly greater at all rates of the MnO and $MnSO_4$, at the two highest rates of the γMnO_2 and the highest rate of the MnEDTA.

TABLE III

Comparison of Fe/Mn ratios in the stem and leaves (P) and in the fruit (F) by rates and sources of manganese applied in pots with Perrine marl

Rate as MnO per acre		Manganese sources			
		MnO	γMnO_2	$MnSO_4$	MnEDTA
10 pounds	P	8.63 N	8.29 O	3.09 N	10.7 D
	F	4.94	7.08	3.21	16.3
20 pounds	P	—	—	—	7.82 D
	F	—	—	—	8.30
40 pounds	P	4.56 N	7.92 S	3.30 N	—
	F	4.14	8.44	2.76	—
80 pounds	P	3.49 N	5.43 N	5.31 N	3.89 S
	F	3.47	5.91	2.44	7.36
120 pounds	P	3.17 N	5.56 N	1.12 N	2.25 N
	F	3.44	5.50	2.01	2.07
L.S.D. 0.01	P	5.30	7.00	5.87	2.16
	F	5.20	4.83	1.68	5.43
L.S.D. 0.05	P	4.12	4.23	3.56	1.31
	F	3.26	3.03	1.01	3.40
No manganese	P	19.6 V			
	F	10.7			

Comparison of the Fe Mn ratios is shown in Table III. With the exception of the MnEDTA at the lowest rate, all the Fe/Mn ratios are highly significantly lower than those of the very deficient plants. With the exception of the plants at the lowest rate of the MnO and the 80-pound rate of the MnEDTA, the Fe Mn ratio in the stem and leaves of plants not showing Mn-deficiency ranges from 1.12 to 5.31 compared to 7.8 or above found in the plants exhibiting Mn-deficiency. The Fe Mn ratio of the fruits from Mn-deficient plants is 7 or greater compared to a ratio of 5 or less from Mn-sufficient plants. Between sources the γMnO_2 has the least effect on lowering the Fe/Mn ratios.

Data from tomatoes grown on Everglades peaty muck

The phosphate levels had no statistically significant effect on the yields, the manganese content and iron content of the tomato plants, or of fruit grown in the outdoor pots containing Everglades peaty muck. The values in the subsequent tables are the average of 4 plants from 4 pots. In Table IV, the fresh weight of the fruit is shown but is not significantly affected by the manganese treatments. The dry weight of leaves and stem is greatly increased at all rates and sources over the Mn-deficient plants. With increasing

rates of the MnO, the weight increased. With the γMnO_2 at the third rate and with the $MnSO_4$ at the second rate, the greatest weight is found (Table IV).

TABLE IV

Comparison of manganese sources in Everglades peaty muck. Average dry weights of stem plus leaves (P), weights of fresh fruit (F) and least significant differences are shown in grams

Rate as MnO per acre		Manganese sources		
		MnO	γMnO_2	$MnSO_4$
20 pounds	P	48.8 D	—	55.4 N
	F	385	—	358
40 pounds	P	49.3 N	49.7 D	59.3 N
	F	352	297	312
80 pounds	P	52.7 N	60.8 S	56.9 N
	F	314	300	337
160 pounds	P	59.8 N	43.1 N	—
	F	364	338	—
Between treatments				
L.S.D. 0.01	P	1.25	—	No manganese
L.S.D. 0.05	P	0.93		P 38.5 V
				F 322 V

TABLE V

Comparison of manganese sources in Everglades peaty muck. Average manganese intake into stem and leaves (P), the fruit (F) and least significant differences between treatments are shown as micrograms Mn

Rate as MnO applied per acre		Units P_2O_5 applied	Manganese sources		
			MnO	γMnO_2	$MnSO_4$
20 pounds	P	80	682 S	—	977 N
		400	640 D	—	1440 N
	F	80	102	—	130
		400	77	—	66
40 pounds	P	80	632 N	408 D	1350 N
		400	649 N	486 D	1270 N
	F	80	81	45	175
		400	103	32	162
80 pounds	P	80	747 N	558 S	1310 N
		400	777 N	534 S	1150 N
	F	80	125	67	144
		400	112	122	122
160 pounds	P	80	1200 N	976 N	
		400	1320 N	985 N	
	F	80	170	158	
		400	170	110	
No manganese	P	80	268 V	L.S.D. 0.01	P 247
		400	252 V		F 85
	F	80	38	L.S.D. 0.05	P 183
		400	48		F 63

The manganese intake of the plants and fruit in this experiment is shown in Table V. The magnitude of the manganese intake was alike at both phosphate levels. At the highest rate of the MnO, intake of manganese by stem and leaves is indicated to be alike that found at all rates of the $MnSO_4$. However, at the first rate of the γMnO_2 the intake was not significantly greater than that in the check plants. The highest rate of the oxides gave signficantly greater intake of manganese than the lower rates. The manganese in the fruit (Table V), shows significant changes which are dependent on both the weight of fruit and the kind and amount of the manganese that was applied.

TABLE VI

Comparison of manganese sources in Everglades peaty muck. Average Fe/Mn ratios of stem and leaves (P), of fruit (F) and least significant differences between treatments are shown

Rate as pounds per acre		Manganese sources		
MnO	P_2O_5	MnO	γMnO_2	$MnSO_4$
20 pounds	80 P	6.41 S	—	9.45 N
	400	10.5 D	—	9.29 N
	80 F	7.71	—	5.47
	400	6.37	—	3.41
40 ounds	80 P	8.97 N	7.37 D	5,15 N
	400	10.1 N	10.7 D	6.41 N
	80 F	5.42	14.6	3.46
	400	6.29	16.3	4.30
80 pounds	80 P	14.1 N	2.21 S	2.96 N
	400	7.54 N	4.57 S	2.96 N
	80 F	3.70	8.39	2.78
	400	3.60	7.73	2.38
				No Mn
160 pounds	80 P	8.31 N	3.20 N	21.7 V
	400	4.84 N	6.77 N	23.9 V
	80 F	3.34	3.66	16.1
	400	3.56	6.51	14.0
L.S.D. 0.01	P	4.84	4.28	6.71
	F	6.31	6.90	6.20
L.S.D. 0.05	P	3.43	3.03	4.75
	F	4.48	4.88	4.40

In Table VI, the Fe Mn ratios show that the rate of manganese is a highly significant factor. All the manganese applications give a decrease in the Fe/Mn ratio in the stem and leaves below that found in the Mn-deficient plants. The Fe/Mn ratio of the fruit decreased also except at the lowest rate of the γMnO_2. Phosphate

levels do not significantly change the Fe/Mn ratios. This indicates that intake of both iron and manganese were not affected by the level of the phosphate supplied. The lowest rate of the $MnSO_4$ has higher Fe/Mn ratios than the other two rates and a similar situation is found for the γMnO_2. However, the MnO source does not show this decrease nor is it shown in the Fe/Mn ratios of the fruit.

TABLE VII

Effect of rates of MnEDTA in Everglades peaty muck for data from analysis of stem and leaves (P) and of fruit (F)

Rate per acre as MnO		Weight grams	Manganese intake micrograms Mn	Fe/Mn ratio
None	P	55.7	348	12.0 D
	F	500	104	8.41
10 pounds	P	77.0	573	5.97 D
	F	496	98	8.40
20 pounds	P	91.7	660	10.3 D
	F	542	65	9.35
40 pounds	P	81.9	1060	5.76 D
	F	444	68	12.3
80 pounds	P	81.5	1100	4.20 S
	F	564	44	24.3
L.S.D. 0.01	P	16.6	151	2.42
	F	N.S.	84	8.21
0.05	P	11.9	108	1.73
	F	N.S.	60	5.87
40 pounds $MnSO_4$	P	90.2	825	8.31 N
	F	569	110	5.56

The comparison of the rates of the MnEDTA is considered separately as shown in Table VII because the experiment was conducted using 2 plants per 4-gallon pot. These plants all showed some degree of Mn-deficiency, whereas the application of $MnSO_2$ gave apparently Mn-sufficient leaves. Although the weights of the fruit are alike between treatments, there is shown to be significantly greater weight of the stem and leaves where manganese was supplied (Table VII). Manganese intake of the stem and leaves is significantly greater over the check plants and the two highest rates of MnEDTA were more effective than the two lowest rates of this source or the rate used of the $MnSO_4$. These observations do not hold for the manganese found in the fruit (Table VII). The Fe/Mn ratio of the leaves and stem shown in the table is higher in the plants not receiving manganese fertilization and lowest at the

highest rates of the MnEDTA. In the fruit, however, an increase is indicated in the iron intake at the highest rate of MnEDTA which resulted in a high Fe/Mn ratio.

Data from tomatoes grown on Scranton sand

The purpose of the experiment using the unlimed and heavily limed Scranton soil was to study the availability of manganese and iron as affected by the liming, by high phosphate level, by the manganese sources and by the rates of manganese applied. The chemical data were subjected to statistical treatment. In Table VIII

TABLE VIII

Analysis of variance of tomato leaves, stems and fruit grown on the Scranton sand

Aanalyses	Source of variation									
	L	P	R	S	LP	LR	LS	PR	PS	SR
Leaves, dry weight	**	—	—	*	—	—	—	—	—	—
Stems, dry weight	**	**	—	—	—	—	—	—	—	—
Fruit, fresh weight	—	—	—	—	—	—	—	—	—	—
Leaves, ppm Mn	**	*	**	—	—	*	—	—	—	—
Stem, ppm Mn	**	—	**	—	—	—	**	—	—	—
Fruit μg Mn/100 g	**	—	**	**	—	**	**	—	—	—
Leaves, ppm Fe	**	—	**	—	—	—	—	—	—	—
Stem, ppm Fe	**	*	—	*	*	—	—	—	—	—
Fruit, μg Fe/100 g	*	—	—	—	*	**	**	—	—	—
Leaves, Fe/Mn ratio	**	**	**	**	—	**	*	*	—	—
Stem, Fe/Mn ratio	**	—	—	—	**	—	—	—	—	—
Fruit, Fe/Mn ratio	**	—	**	**	—	—	**	—	—	*
Total intake Mn in leaves	**	**	**	**	*	—	*	—	—	*
Total intake Fe in leaves	**	—	**	**	—	**	*	—	—	**
Total plant intake Mn	**	**	**	**	*	*	*	—	—	*
Total plant intake Fe	**	—	**	—	**	**	—	—	*	**
Fe/Mn ratio whole plant	**	—	**	**	—	*	—	—	—	—

** F value significant at the 1 per cent level of probability.
* F value significant at the 5 per cent level of probability.
— F value not significant.

the statistical significance of the treatments is shown. In each of the analyses of variance there are 30 degrees of freedom for error variance, 1 for liming (L), 1 for phosphate (P), 2 for sources (S) and 4 for rates of manganese applied (R); and the first degree interactions LP, LR, LS, PR, PS, SR have 1, 4, 2, 4, 2 and 8 degrees of freedom, respectively. The sub-divisions of the data are subjected to the analysis of variance to test if there are differences in the source of the variation. To conserve space the complete data are not

shown. Presentation of data below is made on the basis of the statistical evaluation. Average values are given for each significant factor in each sub-division of data under discussion.

The interpretation of the statistical significance found for weight of leaves, stem and fruit is discussed first. Dry weight of the leaves was increased from 28.7 to 40.6 grams per plant by the liming and was significantly less by 2 grams where the MnO was applied. Dry weight of the stems per plant increased from 12.0 to 18.8 grams by liming and an increase of 1.4 grams was found at the higher phosphate rate. Fresh weights of the fruit were not affected by the treatments in this experiment, but this includes fruit with blossom-end rot which increased the variation.

The manganese content of the leaves was decreased from an average of 390 ppm to 70 ppm by liming. An average increase from 200 to 260 ppm Mn in the leaves was found at the higher phosphate level. Rate of manganese applied increased the manganese in the leaves and the interaction of lime and rates was most significant on the unlimed soil at the highest rates. The average manganese content of the stems decreased from 72 ppm on the unlimed to 19 ppm on the limed soil and increased with rates of manganese applied, particularly on the unlimed soil receiving the manganous sulfate. The μg of Mn found per 100 grams fruit decreased from 1.5 on the unlimed Scranton to 0.85 on the limed soil. The manganese content of the fruit increased with the rates of manganese applied, 0.69, 1.06, 1.38, 1.34, 1.70 μg/100 g fresh weight being the average values. For sources, the average values as μg are 1.50 from $MnSO_4$, 1.17 from MnO and 0.83 from γMnO_2. For the fruit the highest rates of the $MnSO_4$ on unlimed soil gave the highest manganese content, which explains the significant LS interaction.

The average iron content of the leaves decreased from 76 ppm Fe on the unlimed soil to 60 ppm on the limed soil. At the highest rates of manganese the ppm Fe in the leaves decreased, the average for increasing rates being 77, 69, 71, 62, 62. For the plants grown on unlimed soil the stems averaged 50 ppm Fe compared to 35 ppm Fe for the stems from the limed soil. There was a significant decrease in average iron content from 45 to 40 ppm at the higher phosphate level. There was about 7 ppm less iron found in the stems where the γMnO_2 source was used than the average for the other sources. Also the stems grown on the unlimed soil at the lower phosphate

level had the greatest iron content. In the fruit grown on limed soil the average μg Fe per 100 grams of fresh weight was 1.90 compared to 1.02 from the unlimed soil. The iron content of the fruit was greatest on the limed soil at the higher phosphate level. The MnO applied at the rate of 40 and 80 pounds per acre to limed soil gave the greatest iron content of the fruit. Another significant factor was that the iron content of the fruit was much greater where grown on the limed soil fertilized with either the MnO or γMnO_2 sources than where $MnSO_4$ was added to the limed soil.

The Fe Mn ratios of the leaves showed liming to be a highly significant factor (Table VIII). The average from plants on unlimed soil was 0.21 compared to 0.97 on the limed soil. The significant but lesser effect of phosphate gave a Fe Mn ratio of 0.62 for the lower phosphate level compared to 0.52 for the higher level. The Fe Mn ratio of the leaves decreased from an average of 0.70 where no manganese was applied to 0.53 at the highest rate of manganese applied. Where the γMnO_2 source was used the Fe/Mn ratio was greater than for the other sources. As the rates of Mn-application increased, the Fe Mn ratio of the leaves grown on limed soil was less. The greatest difference in the Fe Mn ratio of the leaves between plants grown on unlimed and limed soil was found for the γMnO_2 source and between phosphate levels at the highest rate of manganese applied. The Fe Mn ratios of the stems averaged 3.17 where the soil pH was 7.5 and 0.79 where the soil pH was 5.0, and averaged 3.82 for the higher phosphate level on limed soil. The Fe/Mn ratios of the fruit averaged 1.59 from plants grown on the limed Scranton compared to 0.77 from the unlimed soil. The average Fe/Mn ratio of fruit on the soils not receiving manganese was 1.60 and with increasing rates of application of manganese the ratio was 1.18, 1.27, 1.07 and 0.89, respectively. Average Fe Mn ratios of the fruit where $MnSO_4$ was added gave 0.86 compared to 1.22 for the MnO and 1.47 for the γMnO_2. The Fe Mn ratio was increased more by liming where the oxide sources were used instead of the manganous sulfate. In the fruit there was also an interaction of sources and rates whereby the Fe Mn ratio was least at the highest rates of the $MnSO_4$ application.

Total intake of manganese by the leaves represents about 90 per cent of the manganese found in the plant exclusive of the roots. Interpretation of the sources of variation of manganese found in the

leaves (Table VIII), follows for each statistically significant treatment. The average manganese intake in the leaves by plants grown on the unlimed Scranton was 5800 μg Mn compared to 1400 μg Mn for plants grown on the limed Scranton. At the lower phosphate level the average intake of the leaves was 3000 μg Mn compared to 4200 μg Mn at the higher level. At the rates of manganese applied, 0, 10, 40, 80, 120 pounds calculated as equivalent to MnO, the corresponding average leaf intake per plant was 2410, 3440, 3200, 4160 and 4840 μg Mn. The intake totals from the manganous oxide and gamma manganese dioxide sources were alike and significantly less than that found using the manganous sulfate. Average leaf intake per plant representing the interaction of lime and phosphate gave for the lower rate of phosphate on unlimed soil 4760 μg Mn compared to a value of 1290 μg Mn on the limed soil, and for the higher phosphate on unlimed soil 6880 μg Mn compared to 1560 μg Mn in plants on the limed soil. The highest intake of manganese was found for the leaves from plants on the unlimed soil using the $MnSO_4$ at the higher rates which accounts for the significant LS and SR interactions (Table VIII).

The total intake of iron by the leaves was about 70 per cent of the total sum found in the leaves, stem and fruit. From Table VIII the pattern of significant sources of variation is somewhat different than for the manganese intake. There was a like intake of iron at the two levels of phosphate application. Average iron intake in the leaves per plant grown on the unlimed Scranton was 2150 μg Fe compared to 2460 μg Fe for plants grown on the limed soil. At the increasing rates of manganese applied the corresponding iron found in the leaves averaged 2380, 2320, 2410, 2160 and 2170 μg, respectively. Iron intake was greatest where the γMnO_2 source was used. The significant LR, LS, and SR interactions (Table VIII), are attributable to the decreased iron intake found in leaves from plants grown on unlimed soil supplied with the two highest rates of manganous sulfate.

The sum total of manganese intake per plant, excluding roots, is shown in Table IX. There was consistently greater manganese intake from the unlimed soil for the three sources at all rates. The increased manganese intake at the higher level of phosphate application is also highly significant statistically (Table VIII). A greater manganese intake occurred where the manganous sulfate

was used than where the oxide sources were used. The effect of rates of application on manganese-intake was significant at the highest rates. From the statistical analysis for the SR interaction, the intake at the highest rates of the $MnSO_4$ was greater than for the other Mn-sources. This also explains the significant LR interaction. The LS interaction gives a significantly greater manganese intake for the manganous sulfate on the unlimed soil than other interactions of lime and source of manganese applied. The LP interaction is significant only for the unlimed soil and emphasizes the increased intake of manganese at the higher phosphate on the unlimed soil.

TABLE IX

Total intake of manganese per plant expressed as milligrams Mn							
Rate per acre		Manganese sources					
MnO	P_2O_5	$MnSO_4$		MnO		γMnO_2	
lbs.	lbs.	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed
0	60	6.96	2.90	7.11	3.20	6.76	3.58
0	300	10.87	2.95	8.84	2.53	9.45	2.44
10	60	9.72	3.06	13.90	2.92	8.50	2.86
10	300	13.18	3.96	14.28	3.10	14.21	2.69
40	60	9.65	2.93	10.40	3.66	10.31	2.15
40	300	14.17	3.41	12.44	3.80	12.69	2.80
80	60	14.50	3.33	10.21	3.39	10.40	2.98
80	300	28.90	4.83	12.97	3.97	12.57	3.44
120	60	20.71	3.60	10.67	3.70	10.18	3.03
120	300	37.65	6.81	12.36	5.14	15.72	4.92

The corresponding total intake of iron per plant and the Fe/Mn ratios are given in Table X. A greater intake of iron occurred where the soil was heavily limed than on unlimed soil. This might be explained by the fact that the plant weight was much greater on the limed soil. Level of phosphate applied and source of manganese used did not significantly affect the total intake of iron (Table VIII). The iron intake at the two lowest rates of manganese was greater than at the two highest rates. The LP, LR and SR interactions were highly significant (Table VIII), and the values found at the two highest rates of the manganous sulfate and at the highest rate of the MnO source were responsible for these differences. There was a depressed intake of iron shown at the higher phosphate level where manganous sulfate was used and this might be attributed to higher manganese intake, particularly at the highest rates where $MnSO_4$ was employed.

TABLE X

Total iron intake averaged per treatment as milligrams Fe and average Fe/Mn ratio shown in parentheses

Rate	Manganese sources					
Pounds MnO per acre	$MnSO_4$		MnO		γMnO_2	
	Unlimed	Limed	Unlimed	Limed	Uúlimed	Limed
0	3.27	4.16	3.33	3.55	3.42	3.72
	(0.387)	(1.42)	(0.419)	(1.26)	(0.426)	(1.28)
10	3.41	3.67	3.09	3.89	3.19	3.30
	(0.305)	(1.05)	(0.219)	(1.29)	(0.296)	(1.19)
40	3.67	3.95	2.99	4.08	2.70	4.25
	(0.324)	(1.24)	(0.264)	(1.58)	(0.240)	(1.74)
80	2.58	3.29	3.07	3.39	3.15	3.66
	(0.133)	(0.879)	(0.273)	(0.914)	(0.279)	(1.19)
120	2.71	3.71	2.57	3.44	3.50	4.21
	(0.108)	(0.777)	(0.227)	(0.855)	(0.283)	(1.10)

The Fe/Mn ratio derived from the total manganese and the total iron intake from this soil has a different pattern of variation in some respects from that found in the leaves, stem or fruit (Table VIII). The combination of factors involved show that liming had the main influence. The ratio was at least twice as high in the plants grown on the alkaline Scranton than in those grown on the unlimed soil. Phosphate level was not a factor at the 5 per cent level of probability. Rates of manganese application depressed highly significantly the ratio at the lowest rate over the check plants and the two highest rates depressed the Fe/Mn ratio at the 1 per cent level of probability. The Fe/Mn ratio was significantly greater where the gamma manganese dioxide was used than where other sources of manganese addition were used. Ar the two highest rates the effect of liming had less influence on the Fe/Mn ratio than at the other rates, all three sources being considered.

DISCUSSION

According to Jones and Leeper[5]) the degree of surface disorder of manganese oxide sources is probably the most important characteristic necessary for availability to plants. Hydroquinone extractions of both the manganous oxide and gamma manganese dioxide sources employed in the present studies gave lower amounts of extractable Mn than their synthetic manganous

manganite, manganite, cryptomelane and pyrolusite but higher than their bixbyite and hausmannite. Undoubtedly the particle size distribution is a controlling factor in the degree of these differences. The Auckland ore used in their studies containing γMnO_2 and cryptomelane was reported to be an inefficient source of manganese for oats. Manganosite or MnO to our knowledge has not previously been tested nor has the African ore used in these studies.

The inherent weakness in the present pot experiments is the confining of the roots to a limited soil volume and the necessity of harvesting before the fruit ripened. The factors of soil temperature and moisture though alike in this experiment, are unlike those that would be encountered in the field. Within the limits of these experiments the evidence is that both manganous oxide and gamma manganese dioxide are available sources of manganese. The formulation of the chelate MnEDTA is not as available as the oxides or manganous sulfate on the neutral peaty muck or the marl.

Some estimate of the degree of availability is obtained by comparison of the manganese sources at equivalent rates. The deficiency symptoms along with yield data and chemical analyses gave a rather complete comparison. There is evidence in the experiment that the efficiency with which the γMnO_2 is used increases with time after application and rate of application. The manganous oxide compares favorably with manganous sulfate on the Mn-deficient soils and although early availability at the lowest rate tested was sufficient to prevent deficiency symptoms of manganese in the leaves, yet the chemical analyses suggest a higher rate is necessary. The manganese in the leaves and stem from Mn-deficient plants ranged from 4 to 8 ppm compared to 13 or greater on Mn-sufficient plants. The significance of the chemical analyses of the fruit in comparison of manganese availability has been reported [1]). Both leaves with stem and the fruit were found to be useful in evaluating the availability of the manganese materials. The more detailed study with the Scranton soil showed that comparison using leaf, or stem, or fruit analysis, may be made but that the sources of the variation may be a function also of the part of the plant that is studied. The transfer of nutrients from one part of the plant to another may also affect the results obtained.

The drastic liming of the Scranton sand from pH 5 up to pH 7.5 did not decrease the manganese levels in the plant as low as those

found for the Mn-deficient plants grown on the Everglades peaty muck or the Perrine marl. Possibly a further decrease might occur with subsequent crops. The liming did, however, reduce the availability of the applied manganese which indicates that even if excessive manganese application were made to a Mn-sufficient soil, liming could control the manganese uptake.

The uptake of manganese from the oxide sources appears to be less as increasing levels of application than from the manganous sulfate. Where the manganese availability in a soil is not known, this behavior might prove valuable in the prevention of local toxicity from manganese application to soil.

The increase in the manganese content of the tomato plants at the higher phosphate level may be a response to calcium or to localized acidity from the gypsum. The evidence in support of this is the increased growth of plants grown on the limed soil, and the lack of a significant increase in manganese uptake from the peaty muck at much higher phosphate levels. Also, the iron intake was not different at the phosphate levels used in either the Scranton or Everglades peaty muck. From these results it appears that the Fe/Mn ratio in the plant or fruit is governed principally by the manganese uptake. It may be that sufficient iron was supplied in the fertilization to make iron a non-limiting factor. At high manganese intake some reduction in iron intake occurred but higher phosphate level did not suppress this effect. The determination of Fe/Mn ratios in these plants and fruit was a useful guide in evaluation of the manganese availability in the soils used in these studies.

SUMMARY

The availability of manganous oxide, γ manganese dioxide and MnEDTA was compared to that of manganous sulfate at several equivalent rates on a Mn-deficient peaty muck, marl and on Mn-sufficient Scranton sand. Tomato plants grown in pot studies were analyzed for manganese and iron. Mn-deficiency leaf symptoms were in general agreement with the manganese level in the plant and the Fe/Mn ratio. Iron intake and manganese intake showed some evidence of inverse relationships. Manganous sulfate was slightly more available than manganous oxide which, in turn, was more available than γMnO_2 or MnEDTA.

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TECHNIQUES FOR EXPERIMENTS WITH POT PLANTS

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INTRODUCTION

From time to time, attention has been directed to the special problems of experimentation with pot plants in glasshouses (Le Clerg[1]), Youden[2]), Cox and Cochran[3])). It has been pointed out (Federer[4])) that (*1*) the magnitude of the variations encountered in greenhouse experiments often exceeds that observed in field experiments, and (*2*) all too frequently experimenters assume that the variation in the greenhouse is small enough to be neglected. Very few people have attempted to measure the magnitude and distribution of climatic variables in glasshouses but those who have are well aware of the truth of Federer's remarks. The problem of satisfactory experimental design is further complicated by the lack of space, especially useful space, in a glasshouse, compared with out-of-doors.

In the course of the author's investigations into glasshouse design, seed and potting composts, and methods of propagation (Lawrence[5])), it became apparent that standardization of cultural and experimental techniques was essential to satisfactory experimentation bearing on the germination of seeds in pots, pans and seed boxes; the growing-on of seedlings in small to medium sized pots, in soil blocks and in seed boxes; and the growing to maturity of plants in medium to large sized pots — all on glasshouse benches.

THE PROBLEM

For experiments in glasshouses there are three major sources of error affecting the behaviour of the plant, (i) its genetics, (ii) the

climate, and (iii) the experimental methods. These errors obscure, in varying degree, those very features of plant behaviour the experimenter proposes to study. It is true that correct experimental design will enable an estimate to be made of uncontrollable variations 'in order to decide whether the treatment differences can reasonably be attributed to random fluctuation or not' (Fisher[6]). But as Fisher also points out, refinements in experimental technique 'may be important and even essential, in permitting the phenomenon under test to manifest itself'. The object, therefore, of this paper is to deal with the refinements of experimental techniques as an aid to critical, convenient and economic experimentation. Examples will be given of some of the sources of error in glasshouse experiments. The results of applying the techniques described in the present account are given in the paper that follows (Alvey[10]).

GENETICS

Errors arising from differences in plant behaviour due to genetical factors, can be reduced by using inbred or homogeneous material, *e.g.* hybrid maize, tomato, (Lewis[7]). With high genetical uniformity, differences in growth due to treatments are generally more readily seen and measured. If it is necessary to use species which are outbreeders, or strains that are heterogeneous, *e.g.* brassicas, then some uniformity may be obtained by selecting equal-sized seedlings when pricking-out. This may prevent treatment differences from being obscured by gross genetical variance.

CLIMATE

Uncontrolled variations in light, temperature, humidity and air displacement are frequent sources of error in glasshouse experiments.

1. *Light*

Persistent differences in light intensity of up to a hundred foot-candles or more, commonly occur in glasshouses unperceived by, and therefore unknown to, the research worker. Since within limits as wide as 150–1,500 foot-candles growth of most plants is proportional to the intensity of light during unit time, it will be

realised that differences in light intensity within the glasshouse may and do contribute to errors in glasshouse experiments.

The chief causes of light gradients are 'shadows' cast by (*1*)nearby glasshouses, buildings, trees, *etc.*, (*2*) the structure of the glasshouse itself, (*3*) tall plants near the experiment. Shadow from (*1*) can be avoided by good planning and from (*3*) by simply moving the tall plants further away.

Shadow from (*2*) requires consideration. In a moderately long glasshouse orientated in a north-south direction, heavy shadows are cast by the roof structure during the middle hours of the day,

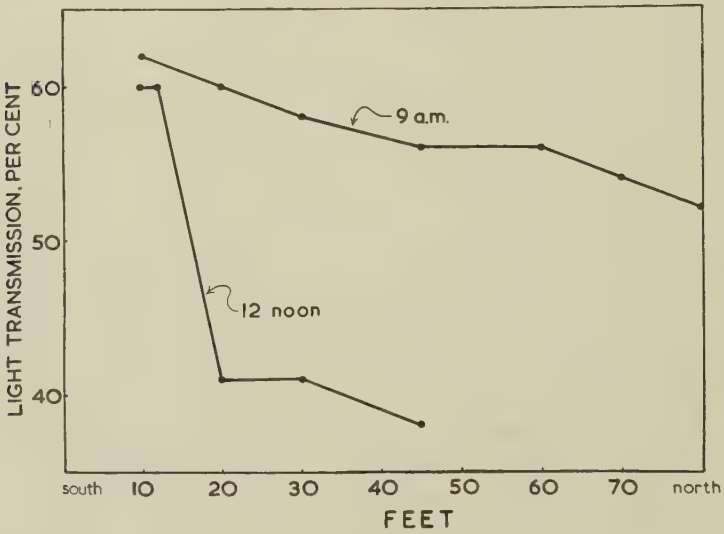

Fig. 1. Showing the light intensity gradients at bench level along the middle of a glasshouse 86 ft. × 20 ft., orientated in a north-south direction. The intensity of light in the glasshouse is expressed as a percentage of the light outside. Measurements made with a Weston selenium cell photometer on 31st December, at Merton.

the shadow being least at the extreme south end. Thus, during a large part of the hours of daylight, a steep light gradient runs northward from the south end of the house, its length depending on the season, *i.e.* the height of the sun (Figs. 1, 2). A similar but less marked gradient often exists even when the sun is not shining, though it is then rarely perceived. Gradients may also occur elsewhere than the south end of a north-south house, *e.g.* across

the house (Fig. 2). The effect of such light gradients on the weight and habit of lettuce can be seen from Fig. 3.

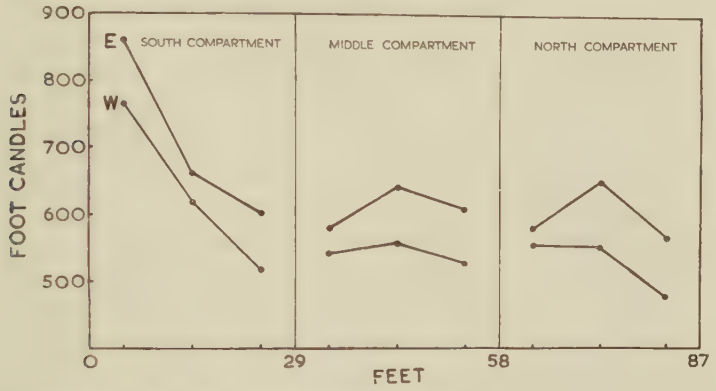

Fig. 2. Showing the light intensity gradients at ground level in the east member of a pair of 'ridge and furrow' glasshouses, orientated in a north-south direction. The house is divided transversely into three compartments by glass partitions. A path runs down the centre of the house. E = the eastern border, W = the western border, adjacent to the second house. Measurements made at 2 hourly intervals, 8 a.m. — 4 p.m., from 8th to 28th October, at Merton.

Most of the difficulties associated with a north-south orientation disappear when the orientation is east-west (Fig. 4). In winter time,

Fig. 3. Showing weights and quality of lettuce 5B grown as a winter crop in the glasshouse referred to in Fig. 2.

in particular, the greatest uniformity in light intensity is found in the single east-west house with high eaves, glazed with large

glass supported on a slender structure with no obstructions to the south of the house and to the north no other glasshouse nearer

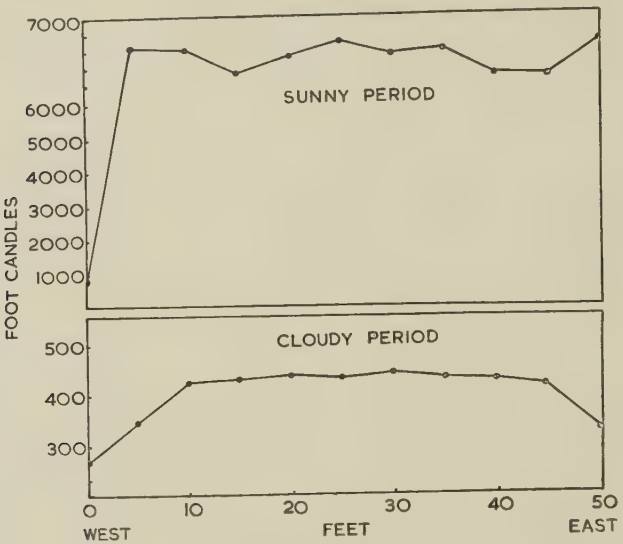

Fig. 4. Showing uniform distribution of light intensity in a glasshouse with east-west orientation. Measurements made on two occasions, both at 3 p.m., during the last week in August. To save space, the foot-candle intervals (ordinate) have been contracted at the lower intensities.

than 20 ft. or so. Such a house is comparatively free of light gradients except at the extreme ends and these can easily be avoided in the lay-out of experiments.

2. *Temperature*

Horizontal gradients in the ambient temperature of glasshouses almost always occur; and sharp gradients have been found to occur on benches within so little as 3 to 5 feet (C o x and C o c h r a n [3]). One such gradient was observed in the course of temperature measurements in a propagating house at Bayfordbury. Two thermometers were suitably exposed 4 in. above the middle of a bench $3\frac{1}{2}$ ft. wide and 48 ft. long, at distances of 19 and 29 ft. from one end of the bench. The mean minimum temperatures over two successive periods of three weeks in October–November were found to be 58.0°F and 56.1°F, and 56.7°F and 54.4°F respectively. Thus a persistent difference of 2°F was shown to exist

between two points only 10 ft. apart, a difference large enough to affect seedling growth appreciably. The cause of this gradient was traced to excess of heat from sub-mains entering one end of the house.

A gradient along the bench is generally the consequence of failure to balance heat losses from the ends of the house by the correct length (surface) of heating pipe. Thus at Merton, where one gable end of a house was fully exposed to wind and the other end protected by an adjoining glasshouse, a mean difference of 5°F in the minimum temperature was found to occur in a distance of 40 ft. Such differences can be corrected by running extra pipes across the exposed gable end and, if necessary, insulating a few feet of pipe at the protected end. In gravity hot water systems the pipe temperature may be 40–50°F lower at the distant end of a glasshouse owing to the sluggish flow of the hot water. The installation of a water circulator is an easy method of rectifying this defect.

Temperature gradients across a glasshouse bench arise from (*1*) proximity to the side of the house, especially where there are ventilators, and (*2*) warm air currents rising from heating pipes beneath the bench and flowing around its edges. In the first case, tight fitting ventilators will reduce the temperature gradient; and the provision of ventilators that come no lower than half way down the side of the house will reduce the gradient still further. In the second case, a clear space of at least 6 in. should be left between the back of the bench and the side of the house to permit warm air to rise from the pipes on the side wall and thus offset the temperature drop near the glass. Even then, for example, in a house controlled at 60°F the mean temperatures at 7 in. and 1 in. from the glass may be of the order 57°F and 53F° in winter time. Careful positioning of the heating pipes beneath the bench will help to reduce edge effects.

The temperature gradient across a 6 ft. wide middle bench is likely to be smaller than that across a 3 ft. wide side bench. Vertical gradients are, as a rule, of little consequence to bench plants. Broken, slipped or cracked panes of glass anywhere in the house are potent causes of local differences in air temperature. Repairs should be immediate and thorough. Equally, ill-fitting ventilators should receive prompt attention.

Once temperature gradients have been reduced to a minimum, maintenance of a uniform air temperature (so far as artificial heat is concerned) can be achieved by means of a thermostat. An efficient heating system should respond quickly to changes in glasshouse temperature induced by solar radiation, wind, *etc.* Electricity, steam, rapidly circulated hot water in 1–2 in. pipes, all give a rapid response to changes in air temperature. A satisfactory thermostatically controlled heating system will, at any given moment, give the required temperature within $\pm$ 2°F and, over periods of hours or months, a very close agreement to the set mean. For example, during one winter, with the night thermostat set at 60°F, the mean night temperature was 59.9°F ($\sigma = 0.70$°F). Similarly, in another glasshouse, the mean was 59.5°F ($\sigma = 0.66$°F).

It should be especially noted that good temperature control necessitates good installation of thermostats and thermometers. Errors of 5°F–10°F have been found to be common on sunny days unless the instruments are adequately screened from direct solar radiation. The larger instruments, *e.g.* thermographs, are particularly unreliable. It is also necessary to ensure that thermometers are adequately aspirated, *i.e.* they must be spaced an inch or so from both screening and supporting materials so that air can move freely past the bulb or sensitive part.

3. *Humidity*

Humidity is difficult to control in glasshouses. Fortunately, where temperature gradients have been minimised the only humidity gradients likely to upset experiments are those created by plants themselves when grown in number on a bench or in the ground. Differences in seedling growth which might arise from these local humidity conditions can be overcome by (*1*) avoiding overcrowding of the plants, (*2*) selective "damping-down" of those parts of the bench which frequently dry out *).

4. *Ventilation*

Ventilation on the lee side of the house and a careful watch for sudden changes in the direction of the wind will help to reduce draughts. But on hot, windy days it is impossible to cool a glass-

*) A 1-in. layer of $\frac{3}{8}$-in. graded and weathered clinker on the bench will greatly help to secure and maintain useful humidity conditions.

house adequately by ventilation alone. Tiffany (cotton), cane, lath or plastic blinds, external to the house, are essential for reducing the effects of sunshine and for making ventilation more manageable. A blind that reduces light to 30 per cent of the outside intensity has been found to be satisfactory.

For cooling purposes, fan ventilation or semi-air-conditioning are inadequate (and costly) for any but quite small glasshouses (or compartments) and even then blinds may be necessary in bright, sunny weather.

EXPERIMENTAL METHODS

These relate to the handling of materials and plants in the preparation of experiments, to the day-to-day care and cultivation of the plants and to the measurements on which the results are based.

1. *General*

i. In preparing experiments certain operations are common to *all* treatments. A given operation should be performed by one man only throughout all treatments, *e.g.* the measuring of bulk compost ingredients, pricking-out, potting.

ii. Ideally, one man should handle the whole of the operations. If, in large experiments, two or more men must be employed, then the principle stated above can be extended, thus. Any operation specific to *one* treatment should be shared equally by the two men.

iii. Standardized methods in definite sequence should be employed consistently so that they become a habit with staff, *e.g.* instruction sheets, prepared labels, measuring of materials.

2. *Composts*

Large variations in plant growth and development may result from the improper making and handling of seed and pottings composts, not only within an experiment, but between successive experiments. Most of this variation can be avoided by using the standardized John Innes Composts. The full details for making these are described elsewhere (Lawrence and Newell[8]). For experimental work the following points are of special importance:—

i. Mixing. This should be very thorough. Fertilizers should first be mixed with some or all of the sand (which must be dry)

and then the fertilized sand thoroughly incorporated with the bulk compost ingredients.

ii. Treatments. When compost is required in two or more lots constituting the treatments to be investigated, uniformity of bulk materials can be ensured by division and sub-division of one original heap of compost.

3. *Pots and boxes*

Clay pots differ in shape, capacity and porosity from one maker to another. These differences may introduce errors in plant growth, therefore pots should be uniform in a given experiment. Thus, in a comparison between two makes of pot of the same size, the weight of one month old tomato plants was 8 per cent greater ($P > 0.001$) with one make of pot than the other. Pots which have been used previously should be thoroughly washed, inside and out, and then immersed in clean water for two days or so. This is a precautionary measure aimed at reducing gross differences in the soluble nutrient contents of the pots. Significant differences in plant weight can arise from the nutrient content of used pots. Glazed pots may be employed, of course, but they are much more costly. Although the author has not investigated variation introduced by previously used boxes, thorough brushing-out is obviously desirable. For the most critical work new boxes should be employed.

4. *Soil blocks*

The chief cause of errors when soil blocks are used is variable soil compression. Uniformity can be secured by seeing that the same amount of soil is fed into the mould for every block. Some machines always make uniform blocks; others require the operator to make adjustments or contrive an even flow of soil to the mould.

5. *Amount of soil*

Weighing or special measuring of the soil can be avoided by using vessels of equal capacity, pouring the soil loosely into the pot or box until it is slightly overfull, then striking-off the surplus soil with a flat piece of wood. The soil may then be compacted if desired. Alternatively, seedlings may be pricked-out straight into the loose soil and watered-in, a method which gives uniform compaction from pot to pot. This is an excellent method with tomato seedlings.

Where crocks must be used for drainage, the most convenient type is the round, slightly convex, perforated metal variety. Potsherds have the disadvantage of requiring sterilization, breaking-up and careful placement. Inevitably, they bulk differently from pot to pot and so alter the volume of soil and, in consequence, the nutrition of the plant. All these disadvantages are avoided by the use of metal "crocks".

6. *Compaction and moisture*

Undue compaction of soil that is too moist, may lead to marked retardation of seedling growth and therefore, to the introduction of avoidable errors. This danger is all the greater if the inherent physical condition of the compost is mediocre. Soil for use in pots and boxes should be in "potting condition", *i.e.* it should just bind or mould when gripped hard in the hand but readily fall apart when the grip is loosened and the soil slightly jolted. If the soil is in potting condition most of the error from differences or compaction will be avoided. With a properly made compost there is no advantage in making the soil very firm. Moderate or slight compaction is enough and this can be obtained by the judicious use of the fingers or by shaped "pressers" which fit the pot or box.

7. *Labelling*

Errors from wrong labelling are obviously among the most serious which can arise since they involve confusion of treatments. If possible, labels should be inserted into pans, pots, *etc.*, before seed is sown or seedlings pricked-out. Preferably, only the one pan or box supplying seedlings for a single treatment should be on the potting bench at a given time and this pan or box should be removed to a distance before the next treatment is dealt with.

8. *Seed sowing*

The aim should be to ensure uniform and rapid germination of the seeds. Incorrect soil texture, compaction, depth of sowing and covering of the seeds are all potential causes of variation in germination and seedling growth. Good texture can be assured by using John Innes Compost which has been passed through a $\frac{1}{4}$-in. sieve. For covering the smaller seeds an $\frac{1}{8}$-in. sieve should be used; finer soil encourages surface panning and poor aeration. Compaction

becomes a danger when the compost is too moist. It is better to have the compost *slightly* too dry than too moist, since it is then difficult to make it too firm, even with intent. Depth of sowing and covering are matters of experience which any competent gardener should be able to deal with. It is better to sow *slightly* too deep than too shallow.

9. *Watering-in*

In the case of small seeds, thoughtless watering-in often leads to variable germination and growth by washing the seeds about and disturbing the surface of the soil. Seed boxes for sowing should be prepared an hour or two before required, well watered with a rose can and then allowed to drain for 1 to 2 hours in a warm place. After the seeds have been sown and lightly covered with sifted soil, just enough water should be applied with a fine rose can to moisten the sifted soil. The box should then be covered with glass, brown paper, *etc.*, to retard drying-out of the soil.

10. *Pricking-out*

Large errors may arise from differences in the way seedlings are pricked-out (or potted-off, or potted-on) by different gardeners (W a r n e [9]). For example, in one experiment with lettuce, the yield from plants planted-out by an experienced man was 13 per cent greater than from those planted by an apprentice (L a w r e n c e [5]). Consequently, it is desirable that in a given experiment the pricking-out, *etc.*, should be done by one and the same individual. If the experiment is too large to permit this, then the seedlings constituting a randomised block should be pricked-out by one man.

It is of outstanding importance, amounting to a principle, that, as far as possible, one and the same person handles and cares for the plants in pot and box experiments. Further, if this person is aware of the part he can play in assisting to establish and maintain a high standard of experimentation, he is worth a very great deal to the research worker. Enlightened co-operation of this kind is a *sine qua non* for intensive experimentation. The research worker cannot always be present to supervise the preparation of materials or the growing of plants and it is surprising how often errors may arise, without his knowledge, through failure of gardeners and

assistants to appreciate the requirements of critical experimentation.

In pricking-out, errors arise from (*1*) the way the seedling is removed from the seed box, (*2*) the care with which it is put into the new soil, and (*3*) the degree to which the soil is compacted by the pricking-out dibber. Errors from these causes can be minimised by (*1*) pricking-out the seedling as soon as cotyledon expansion has occurred, *i.e.* while the radical is still no more than $\frac{1}{2}$ to $\frac{3}{4}$ in. long and therefore, easily removed with the minimum damage, (*2*) holding the seedling by a cotyledon (never the hypocotyl) and ensuring the hole made by the dibber is large and deep enough, and (*3*) using loose-filled pots in which no compaction of the soil follows pricking-out but watering-in is relied on to produce adequate settling of the soil around the seedling root. This latter technique, wherever it can be practised, also has the merit of standardizing the handling of the soil.

11. *Selection of seedlings*

It is sometimes an advantage to select at the pricking-out stage seedlings all of the same size, *e.g.* when the experiment is to test the effect of soil, method of handling, feeding, *etc.* Obvious weaklings and rogues should, of course, be rejected. In other cases, selection cannot be permitted, *e.g.* in genetical studies. In these cases, if all the seedlings are not required, the best method of avoiding conscious and unconscious selection is to take *all* the seedlings from a narrow band or bands, *e.g.* $\frac{1}{4}$ in. wide, across the seed vessel.

12. *Potting-off and potting-on*

These operations are best avoided, since they materially disturb and retard plant development and introduce errors.

13. *The bench*

A "solid" bench or stage covered with $\frac{3}{8}$ in. graded clinker to a depth of at least one inch is preferable to a slatted bench that allows air currents to pass around the plants, thereby introducing variations in growth. The use of clinker, shingle or similar material on a solid bench is not to be recommended when there is a possibility of roots growing out from the bottoms of pots and boxes. Should this happen, large variations in growth may occur. These can be avoided by growing the plants on the bare bench if this is

constructed of rough one inch boards, asbestos-cement sheets, slate, *etc.* Bare boards treated with a preservative harmless to plants are to be preferred to bare slate and asbestos sheet since, in summer time, these mineral materials may become excessively heated by solar radiation to the detriment of plant growth. Further, water evaporates too quickly from a smooth surface such as slate. Rough boards, by comparison, remain moist for a longer time and provide a humid atmosphere around the plants.

At all stages of growth the spacing of pots should be sufficient to prevent the "drawing" which results from overcrowding and the soft growth which is probably an expression of excessive humidity amongst the plants. Spacing in and between the rows should be uniform, though these two spacings need not always be alike. For example, while the requirement for uniform growth might be met by staging young plants pot-thick, the requirements of good plant management (*i.e.* damping-down) might make it desirable to adopt an arrangement whereby the plants were pot-thick in the rows but spaced between them.

It may be noted here that the maximum width for a single bench which permits convenient handling of pot plants is $3\frac{1}{2}$ ft. when the height is not more than $2\frac{1}{2}$ ft. This width allows ten $3\frac{1}{2}$-in. pots to be staged across the bench "pot-thick", or five 5-in. pots, or 4 standard seed boxes. The advantages of the round number ten, are obvious. Alternatively, a row may comprise two lots of five plants. Each row or half row can comprise a plot and n rows a block. It is found that randomised block experiments can be carried out satisfactorily on glasshouse benches.

14. *Watering*

Plants should be watered as uniformly as possible, taking care to avoid both drying-out and over-watering, *i.e.* the ordinary technique of good watering is adequate if applied a little more critically. When plants are to be weighed fresh on a given morning, they should be looked over for watering the previous afternoon or evening so as to have the soil in all pots at "field capacity" and the plants turgid.

15. *Weighing*

In many experiments, especially with young plants, the speediest

method of assessing treatment differences is by weighing the fresh aerial portions of the plant (fresh weight) at a given stage in development. Fresh weight is a reliable criterion of growth when the standardized techniques discussed in this paper are employed. A precaution to be observed concerns the stage at which pot and box plants should be cut and weighed. Partial exhaustion of the soil in respect of one or more nutrients, especially nitrogen, can occur without any obvious signs. Thus, in the case of nitrogen a plant may be a fairly good green colour yet suffering from "invisible malnutrition". This malnutrition will be an intrusive variable in certain experiments, therefore, an effort should be made to terminate the experiment before the onset of malnutrition. If feeding is adopted to counter malnutrition it is desirable to use measured doses of a completely water-soluble fertilizer, freshly made up.

The most suitable level for cutting off seedlings depends on the species. Tomato is best cut off immediately below the cotyledons, lettuce at soil level. For the rapid weighing of single young plants, the author uses a series of fine helical springs suspended in large glass tubes to screen off draughts. For the weighing of groups of plants a useful instrument is a semi-self-indicating balance with an adjustable zero.

DISCUSSION

The techniques described in this paper are essentially simple, calling for no great expense, special apparatus, or highly skilled manipulation. They can be used with profit in many experiments concerned with genetical, morphological and physiological studies of plant behaviour. Thus, in the absence of gross environmental effects, the geneticist can more easily see the direct effect of gene action and interaction and measure genetical differences with greater accuracy. Similarly, the physiologist can compare treatment effects with greater ease and certainty and with economy of time and material.

The development of these techniques places a new experimental method in the hands of the research worker, namely, the statistical assessment of quantitative and qualitative variations in plant growth and development.

CONCLUSION

Good growing facilities are inseparable from good experimentation. This principle is not always recognised in experiments involving the raising of plants in pots and boxes under glass. Commonly the general climatic conditions of the glasshouse are far from ideal with very little control attempted or achieved. And the preparation and carrying through of such experiments is sometimes almost casual so far as uniformity and consistency of technique are concerned. A criterion and standard which has much to commend it may be stated as follows. Good experimentation should satisfy equally the skilled and knowledgeable gardener and the critical and experienced research worker.

SUMMARY

Simple, standardized techniques for the raising of pot plants on glasshouse benches are described. The techniques are especially suitable for the statistical assessment of quantitative and qualitative variations in plant populations by the geneticiest, plant breeder and plant physiologist.

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ERROR IN GLASSHOUSE EXPERIMENTS

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INTRODUCTION

In a previous paper Lawrence[1]) sets out techniques for reducing error in experiments under glass at Bayfordbury. This paper indicates the average size of this error, shows how many replications are needed to obtain results of a stated accuracy and suggests experimental methods of minimising error.

METHOD AND RESULTS

Factorial experiments arranged in randomised blocks are generally employed in this Department (the Department of Physiology and Plant Culture), using the interactions between treatments and blocks as an estimate of error. The method originated by Harris, Horvitz and Mood[2]) enables the average percentage standard error, *i.e.*

$$\frac{\sqrt{\text{mean square for (treatments} \times \text{blocks) interactions}}}{\text{plot mean}} \times 100$$

to be used in calculating the number of plots per treatment required to obtain results of a stated accuracy. In this paper the number of replications have been calculated to give an 80% chance of picking up 10% differences between treatment means at the 5% significance level, which has been found to give results suitable for this Department.

Investigations have been made into pot experiments and germination experiments on the glasshouse bench, and cropping experiments in the borders.

1. *Pot experiments*

The experiments dealt with in this section were carried out in pots on the glasshouse bench to discover the differences produced in plant growth by various cultural methods during the propagation

period, *e.g.* by various composts and various types of containers, and by different light and heat treatments after pricking-out. The effects of the various propagation treatments were calculated on the basis of the fresh weights of plants cut just below cotyledon level after about a month's growth.

In the past, plots containing various numbers of pots each containing a single plant had been in use in the experiments and the number of plants per plot and the percentage standard errors found in each case are set out in Table I*a*. The figures are for tomato (*var.* Potentate).

Fairfield Smith[3]) has shown that the regression of the logarithms of standard error on the size of plot is substantially linear. The log values of number of plants per plot and of percentage standard error, were used to calculate the equation for the regression

TABLE I*a*

Percentage standard errors obtained using various plot sizes in different experiments with tomato *var.* Potentate

No. of plants per plot	Percentage standard error	No. of plots used in experiment
1	24.1	60
1	16.3	60
2	13.6	60
2	18.0	120
4	13.1	48
4	19.8	24
4	9.0	16
5	8.8	32
5	13.6	24
5	19.3	24
5	10.7	24
5	14.5	72
5	14.5	40
5	6.7	40
6	12.1	64
6	10.0	60
6	9.9	60
6	4.7	12
6	15.6	24
8	9.2	50
8	5.5	15
10	6.0	30
10	4.0	28
10	5.5	28

line for the two variables. The log values were weighted by the number of plots used in the relevant experiment in each case. The equation so calculated was:

$$\log \sigma = a - b \log n$$
$$= 1.355 - 0.464 \log n.$$

where σ = percentage standard error and n = number of plants per plot. If the units forming each plot were uncorrelated the value of b would be 0.5. The difference between the value of b and 0.5 was tested and found to be not significant.

The regression coefficient ($b = 0.464$) was based on standard errors and would have to be doubled before substitution in Fairfield Smith's[3] equations where the calculations were based on error variance.

b was also calculated from the results of a uniformity trial consisting of 420 plants pricked-out singly into 3½-in. pots, covering a 7 ft. × 24 ft. bench. As the figures here were not independent, an estimate of b was made from a graph of log plot size plotted against log percentage standard error, bearing in mind that the standard error for the smaller plot sizes would be more accurately determined (Table I*b* and Fig. 1). This gave a value for b of 0.457.

TABLE I*b*

Percentage standard errors for varying plot sizes obtained from results of a uniformity trial with tomato *var.* Potentate

Plot size (n)	log n	Percentage standard error (σ)	log σ	No. of plots
1	0.000	16.91	1.228	420
2	0.301	12.42	1.094	210
5	0.699	8.14	0.911	84
10	1.000	5.84	0.766	42
20	1.301	4.70	0.672	21
30	1.477	4.14	0.617	14

Calculations based on the equation for the regression line were used to draw up Table II which shows plot sizes, percentage standard errors and number of replications required per treatment to obtain the accuracy already stated.

Percentage standard error for various species. The percentage standard errors for various species used in pot

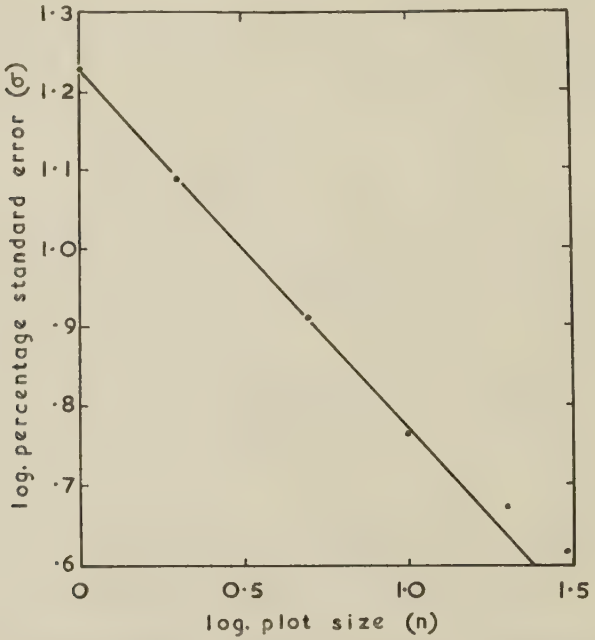

Fig. 1. Showing relationship between plot size and percentage standard error obtained from the results of a uniformity trial conducted with tomato *var.* Potentate.

TABLE II

Numbers of replications and plants required to obtain the stated accuracy with different plot sizes for tomato *var.* Potentate

No. of plants per plot	Percentage standard error	No. of replications	No. of plants
1	22.7	81	81
2	16.4	42	84
3	13.5	30	90
4	11.9	23	92
5	10.7	19	95
6	9.8	15	90
7	9.1	14	98
8	8.6	12	96
9	8.1	11	99
10	7.8	10	100

experiments at this Institution are given in Table III. They are based on plots of five pots each containing a single plant.

TABLE III

Comparison of percentage standard error and numbers of replications required for three species

Species	Average percentage standard error	No. of replications
Tomato	12.6	27
Cucumber	13.4	30
Antirrhinum	21.1	70

Gradients. The results of the uniformity trial were also used to estimate the gradients along and across the bench. There were ten rows running the length of a 7-ft. bench and 42 columns running across it. Table IV shows the figures obtained from this trial.

TABLE IV

Variability of rows, columns and individual plants obtained from the results of the uniformity trial with tomato *var.* Potentate

Source of variance	Mean weight (g)	Variance	Range (g)
Rows	208.60	2.67	190.15–227.20
Columns	49.67	0.85	40.55– 54.85
Plants	4.97	0.64	2.00– 6.70

The differences between rows were highly significant and there was a definite gradient, the plants becoming progressively heavier towards the centre of the bench. The differences between columns were insignificant and no uniform gradient was apparent.

2. *Germination*

The germination experiments on which the following figures are based were carried out to test the effects of various media, *e.g.* sand, vermiculite and soil composts, of the conditions under which such media were stored between mixing and use and of other such cultural methods on germination. Two germination counts were made — the first before all viable seeds had germinated and the second when all viable seeds had germinated. The first count indicated rate of germination. The average percentage standard errors based on experiments using one 6-in pan of 100 seeds per plot and on the germination figures after angular transformation are shown in Table V.

TABLE V

Percentage standard errors obtained in germination experiments with various species				
	1st count		2nd count	
	Percentage standard error	Average	Percentage standard error	Average
Antirrhinum maius . . .	23 7	15	10 7	9
Delphinium ajacis . . .	8 17	13	5 11	8
Nicotiana affinis	10 17	14	7 11	9
Primula sinensis	17	17	10	10

Plot size. As each pan has to be scored there is no point in using more than one pan per plot, although the number of seeds per pan might be varied.

Table VI shows the percentage standard error of several experiments using different numbers of seeds per pan with Antirrhinum. The figures are those for the final count after angular transformation.

TABLE VI

Percentage standard errors obtained when using various plot sizes in different experiments with *Antirrhinum*		
No. of seeds per pan	Percentage standard error	No. of plots
25	11.53	120
25	16.54	16
50	5.28	16
50	11.78	24
50	15.14	24
100	10.08	30
100	6.48	40
150	4.66	16
150	11.14	24
150	6.61	24

The equation for the regression line calculated from these figures was:

$$\log \sigma = a - b \log n$$
$$= 1.489 - .288 \log n$$

where σ = percentage standard error and n = number of seeds

per pan. In each case the log values were weighted by the relevant number of plots for each experiment.

This situation differs from that of the pot experiments, as in these germination experiments plots of equal area but containing different numbers of plants have been used, whereas in the case of the pot experiments the area of the plots was altered also. The value of b still gives an indication of the effect of increasing plot size on the reduction of percentage standard error.

3. *Crop*

Experiments were carried out growing crops of tomatoes in the glasshouse borders to discover the effects on the crop of treatments applied during the propagation stage, *e.g.* variations of temperature and light, or of potting composts. The effects of treatments applied after planting out were also studied, *e.g.* of plant spacings, of manures, or of soil temperatures.

Five plant plots were used in our glasshouse tomato crop experiments and calculations were based on the cumulative totals of ripe fruit picked by the end of each week. The percentage standard error decreased as the total weight of fruit picked increased. Interest was centred mainly on the early crop and it was necessary to discover which was the earliest week's picking on which calculations could be based and the stated standard of accuracy obtained.

Table VII shows the average percentage standard errors, average mean weights and average variance ratios for treatment differences for several weeks' pickings. The figures are based on the results of three experiments for Ailsa Craig and three for Potentate, conducted over the period 1950 53. Results from different experiments were weighted by the number of plots in the relevant experiment. The figures in the Table were used to plot the graphs in Figs. 2 and 3.

At the present spacings about 700 experimental plants can be grown in a 32 ft. $\times$ 102 ft. crop house which is the largest unit that can be spared for one experiment. To obtain the standards of accuracy required, the percentage standard error must not be greater than 20 per cent when there are 350 plants per treatment, *i.e.* in an experiment with factors at two levels, *e.g.* a $2 \times 2 \times 2$ experiment.

TABLE VII

The mean weights of fruit per plot, the percentage standard errors, and the variance ratios for different weeks of picking with two tomato varieties

	Ailsa Craig			Potentate		
Week of picking	Mean weight (ozs.)	Average percentage standard error	Variance ratio for treatment differences	Mean weight (ozs.)	Average percentage standard error	Variance ratio for treatment differences
1st	8.09	66.58	4.68	8.89	108.26	3.09
2nd	29.89	36.97	7.38	43.08	44.91	7.29
3rd	64.50	30.46	3.79	102.83	30.72	8.63
4th	124.60	20.47	6.20	181.98	22.42	8.60
5th	223.69	14.68	5.28	290.31	14.78	10.15
6th	299.71	14.19	4.10	380.93	12.73	8.56
8th	490.81	11.89	3.27	573.36	9.30	8.74
10th	585.70	11.02	2.43	653.35	9.70	5.83

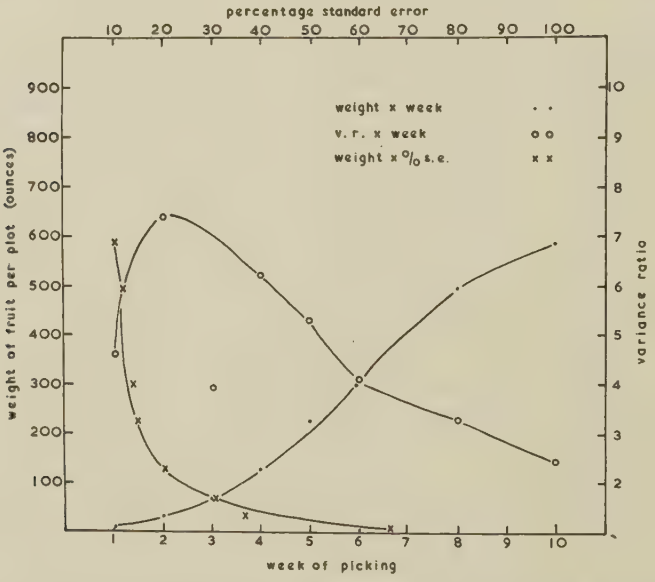
percentage standard error
10 20 30 40 50 60 70 80 90 100
weight x week
v. r. x week
weight x % s.e.
weight of fruit per plot (ounces)
variance ratio
week of picking
1 2 3 4 5 6 7 8 9 10

Fig. 2. Showing the relationships between mean weight of fruit per plot and week of picking, between variance ratio and week of picking, and between mean weight of fruit per plot and percentage standard error for tomato *var.* Ailsa Craig.

From Figs. 2 and 3 it can be seen that the percentage standard error is reduced to 20 when about 130 ozs. of variety Ailsa Craig and about 200 ozs. of variety Potentate have been picked per plant.

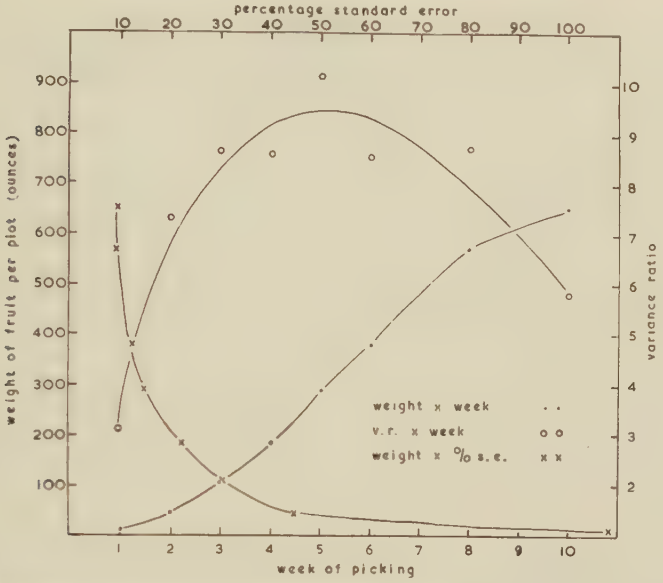
percentage standard error
10 20 30 40 50 60 70 80 90 100
900 800 700 600 500 400 300 200 100
weight of fruit per plot (ounces)
10 9 8 7 6 5 4 3 2
variance ratio
weight x week
v.r. x week
weight x % s.e.
1 2 3 4 5 6 7 8 9 10
week of picking

Fig. 3. Showing the relationships between mean weight of fruit per plot and week of picking, between variance ratio and week of picking, and between mean weight of fruit per plot and percentage standard error for tomato *var.* Potentate.

In both cases this weight of crop is nearly approached in the fourth week.

Gradients. The crop houses are 102 ft. long and 32 ft. wide. They are divided into two compartments (A and B), each about 50 ft. × 32 ft., by a partition. There are two beds each 8 ft. wide in the centre of the house and two beds each $3\frac{1}{2}$ ft. wide at the sides. All beds are divided by 3 ft. wide concrete paths. Two houses have been mainly used for the tomato crop — No. 2, running north to south with an equal span roof facing east and west, and No. 7, running east and west with an unequal span roof, the longer span facing south and the shorter one north. The effects of the paths on the crop picked by the end of the fourth week in both these houses, can be seen in Tables VIII*a* and VIII*b*. The effects of gradients running from east to west and north to south in the two crop houses, on the crop picked by the end of the fourth week, are shown in Table IX.

TABLE VIII*a*

Variation in crop weights picked by the end of the fourth week caused by the effect of paths in house No. 2B

East								West	
3′6″ bed	PATH	8′ bed		PATH	8′ bed		PATH	3′6″ bed	Total
ozs.	ozs.	ozs.	ozs.	ozs.	ozs.	ozs.	ozs.	ozs.	ozs.
1092	1028	442	489	910	580	628	920	817	6906

TABLE VIII*b*

Variation in crop weight picked by the end of the fourth week caused by the effect of paths in house No. 7B

North												South		
3′6″ bed		PATH	8′ bed				PATH	8′ bed				PATH	3′6″ bed		Total
ozs.	ozs.		ozs.	ozs.	ozs.	ozs.		ozs.	ozs.	ozs.	ozs.		ozs.	ozs.	ozs.
1019	979		469	284	297	579		395	316	344	767		341	508	6298

DISCUSSION

The errors incurred with experiments on the glasshouse benches at Bayfordbury are of such a size as to allow satisfactory experiments to be made with reasonable numbers of plants. Growth experiments with tomatoes in small pots can be carried out with about 90 plants per treatment. Whilst there are not sufficient data to draw any definite conclusions about other species used for experimentation at this Institution, the indications are that approximately the same numbers of cucumber and very many more antirrhinum plants would be required to obtain comparable standards of accuracy. The position with numbers of replications required for germination experiments is also satisfactory. It appears that a percentage standard error of about 15 per cent can be expected for the earlier germination count and of about 9 per cent for the final count. This would mean designing an experiment with about 40 replications if the interest lies in speed of germination and with about 15 replications if the interest lies in total germination. These calculations are based on the germination figures after angular transformation.

The factor b (= 0.46) for pot experiments is high and when doubled and substituted in Fairfield Smith's[3]) equation for plot size, shows that it is safe to reduce work by using larger plots containing up to ten plants per plot. Using high numbers of seeds per pan in germination experiments would not appear to

TABLE IX

Variation in crop picked by the end of the fourth week in the two crop houses

House	Year	East side (ozs.)	West side (ozs.)	North side (ozs.)	South side (ozs.)
2A	1950	—	—	4338	3918
	1951	2214	1907	2364	1757
	1952	—	—	3234	3819
	1953	1865	1488	—	—
	1954	3162	3319	3177	3304
Total . .		7241	6714	13113	12798
2B	1950	—	—	5696	3498
	1951	3312	3676	3853	3135
	1952	—	—	2151	1678
	1953	2809	2439	—	—
	1954	3507	3401	3717	3191
Total . .		9628	9516	15417	11502
7A	1951	1420	1682	1684	1418
	1953	—	—	5218	4697
	1954	2540	3319	3443	2416
Total . .		3960	5001	10345	8531
7B	1952	3554	5147	4881	3820
	1953	—	—	3810	3600
	1954	2683	3615	3627	2671
Total . .		6237	8762	12318	10091

offer the same advantages. 50 or 100 seeds per pan are generally used in this Department.

The steeper gradient runs across the width of the bench. The uniformity trial was conducted in winter but the gradient becomes very noticeable in summer and the pots near the edge of the bench require more frequent watering. The gradient is probably due to variation in humidity. Thus, the ideal experimental arrangement would be to run plots across the width of the bench to sample this gradient evenly and run blocks along the length of the bench to abstract the variance due to this gradient. In practice, these two arrangements are irreconcilable on the narrow benches and the best compromise is to run the plots and blocks across the bench. Past experience has shown that this seldom gives a block variance that is significant and it is merely used as another estimate of error.

If the gradients are similar for germination experiments, however, it would seem profitable to run the blocks along the length of the bench. The plots will not sample the gradient across the bench evenly, as each only contains one 6-in. pan.

With the space available at Bayfordbury the design of experiments to estimate early tomato crop should be based on the cumulative crop picked by the end of the fourth week for an experiment with factors at two levels. At this time the percentage standard error has attained reasonable proportions whilst the effects of treatments applied earlier, as indicated by the curve of variance ratio plotted against week of picking, have not been lost. The stated accuracy would not quite be attained from calculations based on four-weeks' crop but if the experimental design were based on five-weeks' crop, only 350 plants would be required and if based on three weeks' crop 1,400 plants would be required. Four-weeks' crop is a reasonable compromise for an experiment with factors at two levels.

Variation in crop picked by the end of the fourth week is caused by the effect of paths and of gradients running from east to west and north to south. It would appear that some form of experimental design that accounts for gradients in two directions, *e.g.* the Latin Square, is the most suitable. As the Latin Square design requires a number of blocks equal to the number of treatments, the blocks would usually have to be small in the limited glasshouse space available. Such a design would be impracticable where large blocks are required as in soil warming experiments. If randomised blocks are used the blocks could be arranged across the width of the house to abstract part of the variance due to the gradient running along the length of the house. The plots could also run across the house to sample the gradient running across the house evenly and the variation due to the effect of paths. This arrangement would be especially suitable in the equal span house No. 2 where the east to west gradient is not so marked.

From these observations it seems that the environmental control in the glasshouses at Bayfordbury is accurate enough to render the differences in plant growth in small pots due to light and temperature over a relatively small bench area, negligible. This does not apply to the larger areas employed in the crop houses where the position is probably further complicated by the fact that the soil in the glasshouse borders is more heterogenous than the potting composts and to ripening of fruit being more variable than plant growth.

SUMMARY

The proportions of plot variances that are assigned to error in glasshouse experiments at Bayfordbury are examined to determine the number of replications required per treatment.

The relationship between average percentage standard error and weight of tomatoes picked by the end of a week is examined to discover which is the most suitable week on which to base an estimation of early crop. This was found to be the cumulative weight of fruit picked by the end of the fourth week after picking commenced.

The gradients apparent on glasshouse benches and in crop houses were studied so that the experimental designs used could take these into account. Randomised blocks are suitable for experiments with small pots and germinating seeds on the benches. Where practicable a Latin Square design would be most suitable for cropping experiments with tomatoes grown in the beds, especially in the unequal span house orientated east and west, at Bayfordbury.

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THE INFLUENCE OF THE ENVIRONMENT ON SEED AND SEEDLING MORTALITY

IV. SOIL TEMPERATURE AND MAIZE GRAIN MORTALITY WITH ESPECIAL REFERENCE TO COLD TEST PROCEDURE

by JOHN L. HARPER and PHYLLIS A. LANDRAGIN

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Maize grains sown in cold damp soil frequently fail to germinate, or the seedlings may die before emergence. Field observations suggest that the extent of this mortality is correlated with the interval between sowing and 50% emergence, and this mortality in turn is correlated with soil temperature. In particular the accumulated hours for which the grains and seedlings are exposed to temperatures below 10°C between sowing and 50% emergence is an important factor (H a r p e r *et al.* [4]). When the soil temperature is controlled in laboratory experiments there is also a marked correlation between soil water content and pre-emergence mortality (H a r p e r *et al.* [5], H a r p e r [3]). The greater part of this mortality is caused by soil-borne pathogenic organisms and evidence has been obtained that the pathogenic activity of the soil population varies significantly with time after allowance has been made for variations in temperature and soil water content (H a r p e r *et al.* [5]).

Varieties of maize differ strikingly in their resistance to pre-emergence mortality, and from early plantings even different harvest samples of one variety or hybrid may give significantly different results in the field. Orthodox germination tests, which are designed to assess the maximum possible germination of a sample usually give no prediction of these differences in field performance. Laboratory tests have therefore been developed which imitate some of the hazards of field conditions, but at the same time allow the conditions to be controlled. Essentially these tests

involve exposing maize grains in soil to low temperatures (usually between 4° and 14°C) for a limited period, after which the grains, with or without the soil, are transferred to conditions suitable for rapid germination. The percentage failure to emerge gives a measure of the liability of the maize grains to pre-emergence blight. Such a 'cold test' has particular value for plant breeders, and also for seed merchants who may wish to test the potency of given seed samples for early sowing. Most maize sold is at present advertised as "cold tested". Wernham [14]) has reviewed cold test methods and some of the results obtained by their use.

The investigations of Dickson [1]) laid the foundation for modern cold testing. The experiments were designed to discover the temperatures at which maize was most severely attacked by *Gibberella zeae*. In these experiments grains of maize were soaked in a conidial suspension of the fungus before they were sown in soil at controlled temperatures ranging from 8–32°C. The soil temperatures were controlled in Wisconsin soil tanks and the grains were subjected to one particular temperature during the whole period of germination and emergence. He was able to show that the most intense "blighting" of corn occurred between 8° and 20°C and none above 24 (see also Dickson and Holbert [2]). Germination did not take place below 8 C and no assessment was made of the residual viability after exposure to lower temperatures.

Gibberella zeae is only one of the organisms which may cause pre-emergence blight, and is probably mainly seed borne. Other soil-borne organisms, particularly species of *Pythium*, are important causes of pre-emergence blight (Richardson [10]) and in order that grains can be tested against the whole complex of soil micro-organisms, cold testing is normally carried out in unsterilised soil from fields in which maize has been grown for one or more years. The use of a whole complement of soil organisms as the source of pathogens in the test carries the great advantage that the type of hazard experienced in the field is closely imitated. The variability of the test soils (Harper *et al.* [5]), however, constitutes a serious weakness in the test as soils cannot easily be standardised except perhaps by reference to the mortality induced in a standard maize sample. Standardisation of the test medium can of course be obtained if pure cultures of pathogens are inoculated into sterile soil or synthetic soil systems to provide the test medium: it is,

however, questionable whether the advantages so gained in ease of standardisation outweigh the disadvantages innate in such an artificial system.

The cold testing procedures employed or recommended by various investigators differ considerably (Wernham [14]). It is obviously desirable that so far as possible these procedures should be standardised so that the results of varietal tests made by different investigators may be compared.

In the experiments described in this paper the aim has been to determine the interaction between four of the variables which have been introduced into routine cold tests.

1. Variations in the apparatus and procedure used in making the test.
2. Variations in temperature of exposure in the cold test.
3. Variations in time of exposure of the cold test.
4. Whether there has or has not been a period of germination at a higher temperature before cold treatment.

The experiments were designed both to compare various cold test techniques and at the same time to gain further knowledge of the general reactions to changes in environmental conditions of the populations of soil organisms responsible for pre-emergence blight.

The influence of these varying factors was determined using two varieties of maize (a) an open pollinated cold sensitive variety — *Virginian White Horsetooth* and (b) a double top cross hybrid of excellent performance in Northern temperate climates — *Wisconsin 275*.

Experimental

1. *Comparison of cold test procedure by the standard soil tray method and the modified Hoppe technique.*

In large scale cold tests in which many varieties are examined simultaneously, there is often a serious shortage of 'cold room' space. Dickson's [1]) use of Wisconsin soil temperature tanks for the maintanance of soil temperature, is extremely expensive on a large scale. If refrigerators are used for the tests then there is clearly a serious limitation of space. 'Walk in' cold rooms have been extensively used for cold testing (Van der Meulen and Henke [13])) but even here problems of space may become acute if the grains are sown in boxes of soil. Hoppe [8]) showed that

this problem might be solved by cold treating the grains in soil in small glass tumblers, and then removing the grains from the soil and planting them in trays of damp sand which were maintained at a temperature suitable for rapid germination. H o p p e reports no tests to determine how the results of this technique differ from the normal practice in which the trays of soil are removed with the grains *in situ* to the higher temperature at the end of the cold period. It would seem likely that the results of the two tests would differ (a) if the conditions of aeration have a marked influence on the damage during the cold test, or (b) if any significant damage is done to the grains by exposure to the soil during the phase of rapid germination.

An experiment was designed to compare these methods. Two varieties of maize were used, White Horsetooth and Wisconsin 275, and two soil types were employed, a light sandy loam (Sandford) and a heavy alluvial clay (Wytham). On both of these soils maize had been grown in the previous year. The experiment was repeated on two separate occasions, in January 1952 and March 1952.

The soil was sampled from the field and sifted through a $\frac{1}{2}$-inch mesh sieve. The sifted soil was used to fill wooden seed boxes 15 inch × 10 inch × 3 inch and in these were planted 24 grains of each variety at a depth of 1 inch. 1-lb. jam jars were used in place of the glass tumblers of H o p p e, and in these were sown 24 grains of each variety, mixed with the soil so that the grains were evenly distributed within the jar. The experiment was prepared with four replicates of both jars and trays, two of which were incubated at 8° C and two at 20° C for 8 days. After these treatments the trays of soil were all incubated at 20° C until no further emergence of seedlings occurred. The grains in the jars were examined after the 8 day treatment and all those which had failed to germinate were cleared of attached soil particles and planted in shallow trays of damp sterilised sand at 20° C (a grain was considered germinated if the coleorhiza had penetrated the pericarp). The number of grains which failed to germinate was determined, see Table I.

Statistical analysis showed that:

(1) there was no significant difference between the mortality induced by the tray and jam jar treatments. This result suggests that no significant damage results from the exposure of grains to

soil at 20°C following the cold treatment, and that this phase of the cold test can, when necessary, be made in sterile sand.

(2) for the two soil types there was no significant difference between the mortalities over both sampling occasions (see Harper, *et al.* [5]).

(3) there was a significant difference between the cold test reactions from the two dates of sampling ($P = < 0.05$) (see Harper, *et al.* [5]).

(4) the two varieties of maize differed strikingly in cold test response ($P = < 0.001$), Wisconsin 275 showing the greater survival under all conditions.

(5) there was a marked difference between the mortality induced by the two temperature treatments.

(6) there was a highly significant interaction between variety and temperature ($P = < 0.001$).

TABLE I

The survival of maize grains after exposure to 8°C and 20°C for 8 days in trays and jam jars of soil, and subsequent germination in soil and sterile sand

Sampling date	Variety	Soil type	Trays		Jars	
			20°C	8°C	20°C	8°C
Jan. 25th, 1952 . . .	W. 275	Wytham	88	78	84	80
		Sandford	80	78	82	78
	W.H.T.	Wytham	68	34	72	38
		Sandford	78	32	72	34
March 19th, 1952 .. .	W. 275	Wytham	94	78	42	40
		Sandford	86	90	90	90
	W.H.T.	Wytham	72	50	72	50
		Sandford	76	54	80	60

It is clear that the results of a jam jar technique are quite similar to those from a tray technique, and so cold tests obtained by various workers using these two techniques may *in this respect* be comparable. The jam jar technique was used in the following experiment.

2. *The influence of variation in temperature, time of exposure and pre-treatment on the mortality of maize grains in soil.*

This experiment was designed to investigate both the influence of temperatures (0°, 3°, 8°, 15°, 20°C) and the period of exposure (2, 4, 6, 8, 10 days). This range would cover many of the temperatures

and length of treatment combinations used in cold test schedules of previous investigators. Some of their schedules include a period of 2 days pre-treatment in which germination is allowed to start before the cold treatment. This experiment included 0 and 2 days pre-treatment at 20°C.

The two varieties of maize and the light loam from Sandford were used. Thus the experiment consisted of the interaction of 2 varieties at 5 temperatures, 5 lengths of exposure, with or without pre-treatment with 2 replicates each of 50 grains of maize.

This experiment involved 200 samples of 50 grains, and the experiment was too large to be handled in a single day. Each grain sample required careful setting in the soil to ensure that the grains were not touching each other or the glass. Also after the period of cold treatment the extraction, cleaning and setting of the seed in moist sand was a lengthy process.

The whole experiment was for this reason divided into five parts, each corresponding to a different period of exposure. The soil sampled on any given date was therefore used for the investigation of the effect of interaction of temperature, variety and pre-treatment on grain and seedling survival. This part of the experiment was repeated five times in order to introduce the influence of time of exposure into the design.

The division of the experiment into parts, however, while making possible an experiment on so many interacting factors, introduced a serious complication. It was clear from the results of earlier experiments (H a r p e r *et al.* [5]), that there are with time significant changes in the pathogenic potential of the soil. The experimental design outlined above, requires that any effect due to different times of exposure includes also any variation in the pathogenic potential of the soil which may have occurred over the period of the whole experiment. (This illustrates the general problem that is introduced into maize cold testing by the discovery of the constantly changing nature of the soils pathogenic potential). For this reason, the experiment was carried out concurrently with the determination of the pathogenic potential of the soil described in an earlier paper (H a r p e r *et al.* [5]), and in this way an indication of the pathogenic potential of the soil was obtained for each part of the experiment. It was not possible however, to use this information as a guide for correcting the data for the five periods of

exposure so as to eliminate the effects of variations in pathogenic potential, because it would have been necessary to know not just the drift of the pathogenic potential at 8°C for 10 days exposure, but also to know the drift over the whole range of temperatures and times of exposure. This was clearly impracticable. Instead it was assumed that there would be a drift in pathogenic potential over the experimental period rather than erratic variations. The five parts of the experiment were therefore randomised over the five periods of sampling, so that if there was a drift, its occurrence would not add to or subtract in any regular fashion from a regression of grain damage on time of exposure. Any change in mortality with time of exposure could not then be ascribed to simultaneous drifts in pathogenic potential, although, of course, error due to such drifts would still be included with error due to time of exposure.

It was found at the end of the experiment that there had been a fairly steady increase in the pathogenic potential of the soil over the whole period of the experiment which lasted 10 weeks. It is clear from Table II that the order in which the parts of the experiment were performed would, in fact, randomise this drift over any progressive effects due to increasing periods of exposure. Any regular change in grain and seedling mortality with time of exposure found in the results of the experiment was therefore almost certainly due to time of exposure.

TABLE II

Sampling date	% Mortality in standard cold test 8°C for 10 days = pathogenic potential	Days of exposure given to divisions of main experiment
Jan. 11th	42.4	2
25th	33.2	6
Feb. 8th	55.1	10
22nd	59.7	8
March 5th	66.6	4

Of the 16 samples of each variety at each sampling date, 8 were distributed between the four temperatures and exposed to these immediately, while 8 received 2 days pre-treatment at 20°C before being similarly treated. After the appropriate period of exposure in the soil, the grains were removed, cleaned free of attached soil

particles and planted $\frac{1}{2}$ inch apart in trays of damp sand at 20°C. Many of the grains had germinated during the longer treatments at the higher temperatures; only grains ungerminated at the end of treatment were planted in sand. The germination of these grains was recorded each day, and the germinated grains were then removed.

When recording the results, it was observed that many of the grains which had not germinated were decaying and had produced considerable growths of fungal hyphae, especially in the case of White Horsetooth. Often fine white hyphae could clearly be seen extending across the sand from one grain to its neighbours. It seemed possible that some of the grains were damaged, after their exposure in soil to low temperatures, by hyphae extending from neighbouring diseased grains in the sand: the results recorded would not then give a true estimate of 'cold' damage. An analysis was therefore carried out of the results obtained from one of the sampling dates, noting the position of each diseased and healthy grain on the tray. The analysis was carried out with respect to the number of pairs of neighbouring diseased grains. The total number of diseased grains was counted, and from the number of positions on the tray which these could occupy, the total expected to occur in pairs could be calculated. This expected value compared with the total observed to occur in pairs gives an indication of whether lateral infection leads to mortality in the trays.

The number of pairs of diseased grains was no greater than that expected to occur by change. It was therefore concluded that lateral infection of fungal growth from one grain to another in sand trays is not a significant factor.

The percentage germination results are recorded in Figs. 1 and 2. The analysis of variance excluding the treatments at 0°C which gave anomalous results to be discussed in a later paper, showed that the effects of variety, temperature and time of exposure were all significant at $P = 0.001$. The first order interaction of temperature and exposure-time was significant at $P < 0.001$ and of variety and temperature at $P = < 0.05$. A comparison of Figs. 1 and 2 shows that for grains which had not been germinated before treatment, White Horsetooth suffered the greater mortality, but that the trend of damage with both temperature and time was essentially the same for both varieties. Two days treatment resulted in little mortality, but four days treatment brought about ap-

preciable damage to White Horsetooth at the lower temperatures. Six, eight and ten days treatment induced correspondingly larger increases in cold damage to both varieties, particularly at 3°C and at 8°C and to a much lesser degree at 15°C.

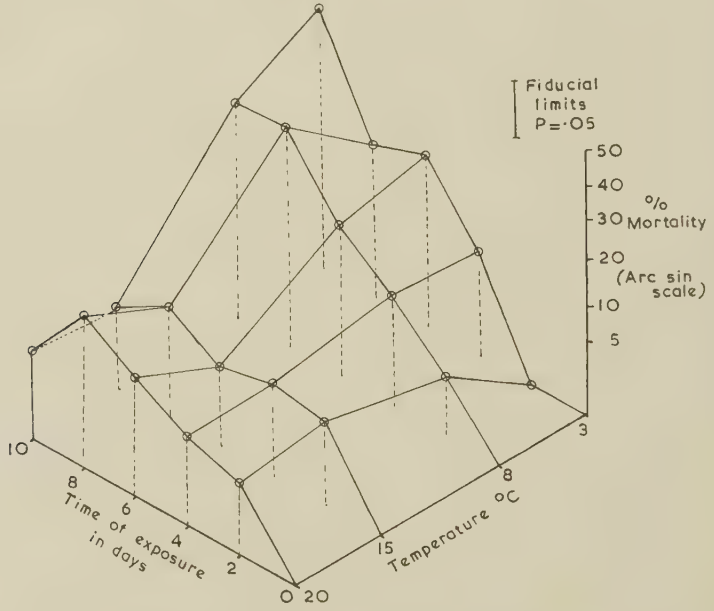

Fig. 1. The percentage mortality of maize grains determined after different periods of exposure in soil kept at various temperatures and without pre-treatment. Variety: Wisconsin 275.

With 6 or more days treatment, the steepest part of the curve relating survival to temperature lay between 8°C and 15°C. Between these values slight variations in temperature result in relatively large changes in percentage survival. A number of cold test procedures, (Pinnell[9]), Rush and Neal[11]) and Wernham[14])) have involved treatment at temperatures between these two values. The results obtained here suggest that temperatures of 8°C or below would be less sensitive to minor variations in conditions of the test.

There was relatively little difference in the mortality sustained at 3°C and 8°C. Haskell[6]) had found 4°C to be consistently less damaging than 10°C while Tatum[12]) found a similar relationship between a temperature slightly above 0°C and 7°C although the relationship between the two temperatures was not

in any way constant, in some cases the mortality was greater at the lower temperatures and in others it was less. At 0°C the degree of damage increased over the first four days, at this stage being greater than at 3°C or 8°C. Over longer periods of treatment however, the mortality at 0°C was not greatly altered as compared with 3°C and 8°C.

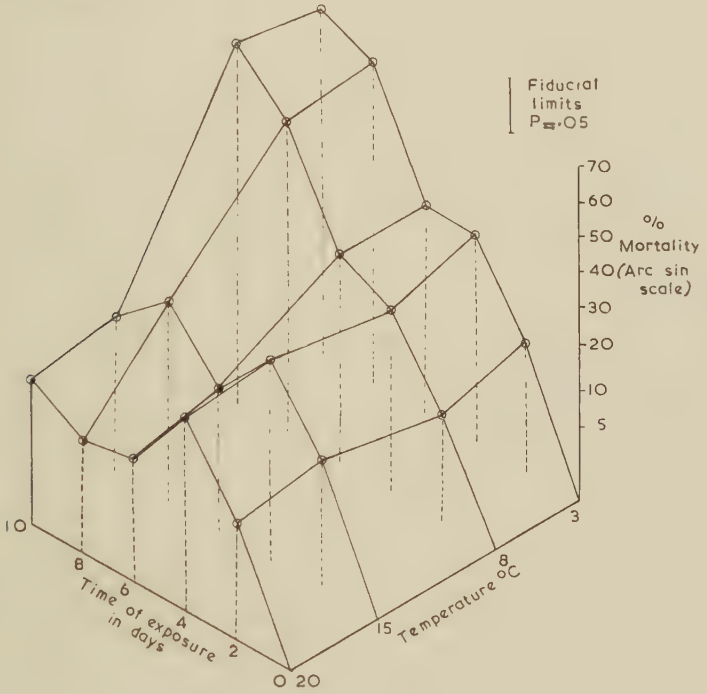

Fig. 2. As fig. 1, but variety Virginian White Horsetooth.

After pre-treatment the majority of grains showed the radical protruding from the pericarp for a length of from 1–5 mm. There is no significant difference between pretreated and non-pre-treated grains in respect of mortality at 3, 8, 15 or 20°C for any length of exposure. The grains exposed to 0°C after pre-treatment gave very variable results. Later experiments have shown that true frost damage plays a part in determining mortality at this temperature and these experiments will be described in detail in a later paper.

DISCUSSION

The inability of maize grains either to survive dormant, or to germinate very slowly in cold wet soils is of direct interest for two main reasons. First it suggests that the successful establishment of the seedlings is largely determined by hazards in the pre-emergence phase, and this in turn suggests that maize may exemplify a group of plants which are geographically or ecologically limited in this way. This mortality is largely due to soil-borne pathogens, and maize varieties differ in their resistance to this cause of mortality. A second point of interest is the possibility of testing maize grains for their ability to survive in cold soils by exposing them to such a hazard in the course of routine germination tests. The many attempts which have been made have not used adequately standardised techniques, and it has already been shown (H a r p e r *et al.* [5]) that variations in the 'pathogenic potential' of the soil associated with changing water content of the soil as well as significant unexplained variations may make the standardisation of 'cold testing' very difficult. The measure of the cold damage produced by 10 days exposure to 5°C in soil, followed by transference of the grains in soil to 20°C, varied from time to time. The experiments described in this paper show that there is considerable variation due to differences in the temperature at which the test is carried out. It is clear that under the conditions of the present experiments there is a very marked difference between the mortality at 3°C, 8°C and 20°C. This difference is most marked between 8°C and 15°C, and in any test carried out between these two limits of temperature there are likely to be errors due to small changes in temperature. Although it is relatively easy to arrange a room or cabinet for cold testing which maintains an even temperature, it is much more difficult to control the temperature of the soils stored within it. Variations in the ambient air humidity may change the rate of evaporation from the soil and so change its temperature. According to the results of this experiment, variations of a few degrees in temperature will have least effect between 3° and 8°C, and it is suggested that a standard temperature for testing should be chosen between these limits.

It is clear that mortality increases with time up to 10 days exposure, and the difference between the two varieties examined

becomes increasingly marked up to 10 days exposure. It is likely that longer exposures would have induced greater mortality and accentuated even more the varietal differences, but a period of 10 days exposure is probably long enough for routine tests. This conclusion confirms the findings of Haskell[6]). Pre-treatment effects no significant change in the mortality at temperatures above 0°C. This contrasts with the reports of Hooker and Dickson[7]), who found an increase in the resistance of excised embryos to infection by *Pythium* with the extent of seedling development. However, this contrast may perhaps be due to the differences between the present test methods and those of Hooker and Dickson, who used excised embryos and pure cultures while in the present experiments whole grains and unsterilised soil were used.

SUMMARY

1. Some of the differences between various techniques of 'cold testing' corn have been examined. No significant difference was found between the results of cold testing by exposing grains to temperatures between 3° and 20°C after 0 and 2 days pre-treatment at 20°C (Erratic results are reported for the mortality induced by exposure of pre-germinated grains to 0°C).

2. Mortality in the cold test increased with time of exposure up to 10 days (the longest period involved in the experiments). Varietal differences between Wisconsin 275 and Virginian White Horsetooth became more pronounced with increased period of exposure.

3. There was relatively little change in mortality between the cold tests conducted at 3° and 8°C compared to the large change between 8° and 15°C. It is suggested that the temperature of the test should be standardised within the lower range, where small uncontrolled variations in temperature will have least effect on the tests. Attention is called to the fluctuation in soil temperature which may be caused by variations in atmospheric humidity in a 'constant temperature' room.

4. Comparison of 'cold tests' made (*a*) in jam jars of soil with subsequent sowing of the grains in damp sand at a higher temperature with (*b*) tests involving grain treated in boxes of soil in which the grains remain *in situ* through all phases of the test, reveal no differences due to these contrasted procedures.

ACKNOWLEDGEMENT

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A NEW METHOD FOR THE SIMULTANEOUS PRESERVATION OF PROFILES AND ROOT SYSTEMS

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Description of profiles has been carried out for many years, but there has remained the difficulty that it is impossible to check the data afterwards by using material collected from these profiles. Since this is often desirable, people have tried to preserve profiles. Preservation, however, is only of value, if the characteristics of the profiles are not altered by the preserving method and if the preserved profiles take up as little room as possible and remain usable for an unlimited length of time. Several authors have tried to meet these requirements. Here we will only mention those two methods which give, in our opinion, the most satisfactory results.

Voigt[7]) described in 1936 a method with which he had obtained good results. In this method a kind of resin (Zaponlack) is applied to the exposed face of a profile in order to fix the soil particles near the surface. After the resin has dried up, the preserved soil film can be removed by hand. This method has been used in the Netherlands during the recent years by Tanis with considerable success [5])[6]). Instead of Voigt's resin solution Tanis made use of a solution of cellulose, which is probably an improvement. This cellulose solution is manufactured by a Dutch factory; its exact composition and concentration are unknown. The solution has a high grade of viscosity, which makes it often necessary to dilute it before application. A mixture of liquids to be used for dilution of the cellulose solution is also obtainable from the same factory.

In 1945 the American investigators Berger and Muckenhirn gave a description of another method [1]). Here use is made of a solution of an artificial resin (vinylite, grade VYHH) in acetone or in methylisobutylketone. We used this method in 1948 [4]).

Preserved profiles nearly 7 years old are still present in a good condition in our laboratory.

Both of these methods have their advantages and drawbacks. The method of Voigt is more simple than that of Berger and Muckenhirn and may give better results with regard to the structure of the soil. On the other hand, in using Voigt's method one is more dependent upon the weather conditions. Rainy weather hampers the preserving effect of the cellulose solution. In general, its applicability is limited, in case of moist profiles, owing to the fact that the penetration of the cellulose solution is reduced in rate by the soil water. Finally it may be a disadvantage, that it is always necessary to visit the place of collection twice, at intervals of at least 10 hours. This is due to the fact, that the preserved soil film can only be removed from the profile when the cellulose solution is dried up completely. Hence this method is rather laborious and expensive. This trouble is of course not so important in the case of mass production of preserved profiles.

When the agricultural value of the profiles to be preserved is to be demonstrated it is important to show simultaneously data concerning the chemical composition of the various layers of the soil, as well as data concerning the moisture conditions.

Usually the yield of a crop may be a measure of the effectiveness of the profile conditions but it is difficult to draw conclusions about the value of the various soil layers without some preservation of plant material. Indications of this are given by the root development. Hence we have developed a method by which the preservation of profiles and of the roots of crops growing on these profiles can be accomplished simultaneously. Kullmann [3]) recently described a method, which gives, however, rather unsatisfactory results. In this communication a description is given of our method.

We started by preserving profiles by the method of Voigt, as modified by Tanis. This led, however, to the difficulties mentioned above. Consequently the method was modified. Initially a partial combination with the method of Berger and Muckenhirn was achieved by using the iron frame of these authors. With this frame monoliths can be taken from the exposed face of a profile. The dimensions of the frame were, in our case, 100 × 25 × 5 cm (Fig. 1) but these dimensions may be varied.

After having obtained one monolith another was taken from the same wall for root investigation by means of a pin-board (Fig. 2). This method is regularly applied at our Station and has been described by Goedewaagen in 1948 [2]). The root system of the crop growing upon the soil surface of the monolith can be uncovered by washing away the soil. The pin-board generally used has a height of about 1 meter, a width of 30 cm and pins of length about 7 cm disposed at distances of about 5 cm. The pins ensure that the morphological structure of the root system remains intact during the washing.

Fig. 1. A. Iron frame (1) with board (2). In the brim of the iron frame the screws are visible with which the board is fixed on the iron frame. B. The iron frame is forced into the soil. On the part, where the board is omitted the brim of the iron frame is visible.

The monoliths in the iron frames, assigned for preservation, are transported to the laboratory for treatment with the cellulose solution. The transportation must be done carefully in order to avoid cracking. It may be possible to preserve several soil films from one monolith in its iron frame. Another advantage of the use

of the iron frame is a greater independence of the weather conditions, because the application of the cellulose solution can be postponed till the return to the laboratory. The preservation of even wet soils is possible in the laboratory by waiting until they are dried to the point, when the solution of cellulose can penetrate into the pores.

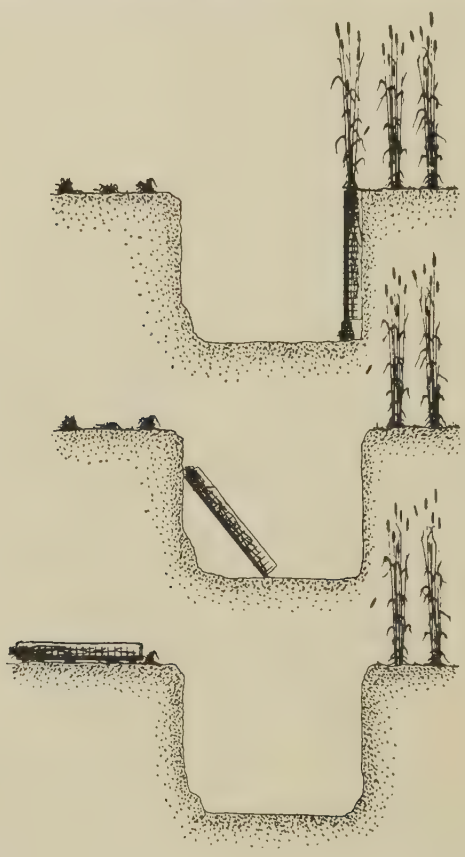

Fig. 2. Scheme of the sampling with a pin-board on behalf of root investigation.

The soil from the monolith on the pin-board is washed away, leaving the root system lying between the pins upon a sheet of black plastic, which is pushed between the pins before the soil sample is taken.

After a little investigation it was shown that the method, described above, can often be simplified by taking one monolith only

on the pin-board. This monolith can be used both for the preservation of a profile and also for uncovering the root system, provided the thickness of the monolith exceeds the length of the pins by about 4 cm (Fig. 3). The advantages are evident. By taking only one monolith for both treatments the preserved profile and the root system are derived from closely adjacent parts of the soil. Consequently the root development on the pin-boards generally shows a close response to the properties of the soil, as indicated by the preserved soil film.

This simple method can only be applied if the pins do not cause the soil to split. Cracking of the soil sample may occur for instance where hardpans are present. In such cases only is the use of the iron frame preferable.

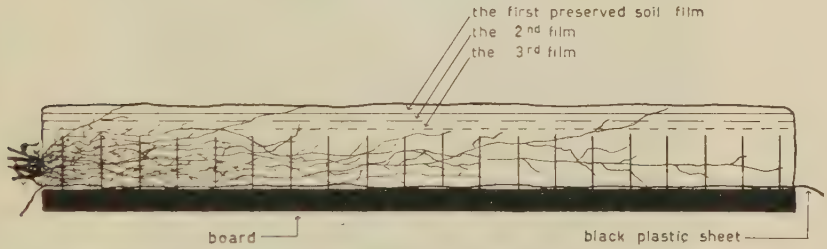

Fig. 3. Scheme for taking one or more soil films and for fixing the root system by means of a pin-board sample.

The actual preservation of a profile on a pin-board in the laboratory is carried out in the following way. The monolith is placed in a slightly tilted position. The surface of the soil outside the pins is smoothed carefully by means of a sharp knife. It may be necessary to sharpen the knife repeatedly during this process. Next the solution of cellulose is poured over the smoothed surface. (Fig. 4), after which the profile is left untouched until the cellulose solution is dried to a firm but flexible film. This can then be removed from the monolith by pulling it away. We start pulling at the bottom of the profile in order to avoid the fixation of loosening soil particles in the wrong place (Fig. 5). Profiles taken on grassland should preferably be cut free in the upper 10 cm since the top layer often contains so many roots the strength of which exceeds that of the cellulose film. Because of the smoothing effect of the knife nothing is visible of the natural structure of the soil in the upper

part of the film. It can, however, be made visible later by tapping the inner side of the prostrate film with a hard brush, so that its natural appearance is again developed.

The thickness of a cellulose film in sandy soils is on the average only a few millimeters.

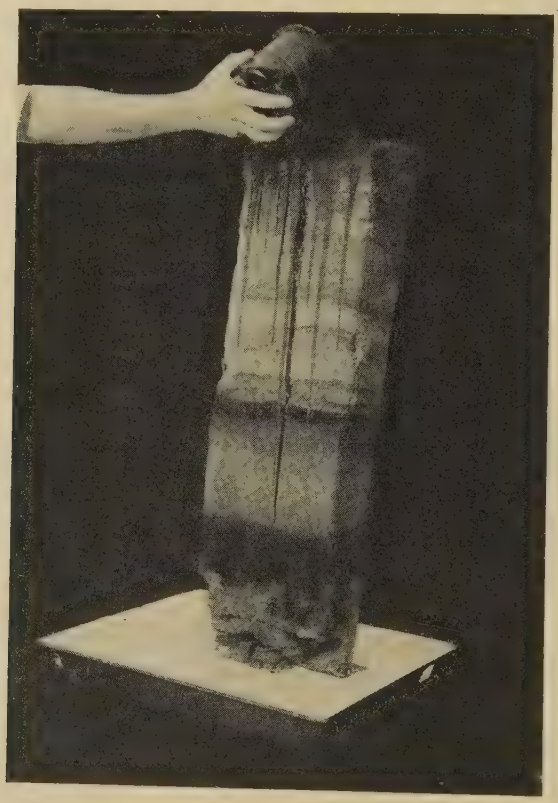

Fig. 4. The cellulose solution is poured over the face of a soil sample on a pin-board.

In some cases preservation of a number of films from one profile may be required. This can be made possible in the following way. After having removed the cellulose film the soil of the monolith is smoothed again and then the whole process may be repeated in the way described above, several times, even after the tops of the pins have become visible (Fig. 3).

When the required number of profiles has been preserved, the

remainder of the soil sample is washed away by means of a stream of water. For this purpose the board with the sample is laid in a wooden box partially filled with water. During the washing the root system drops to the board, from which it can be removed by lifting it up by means of the black plastic sheet lying underneath. Plastic and roots are then dried together.

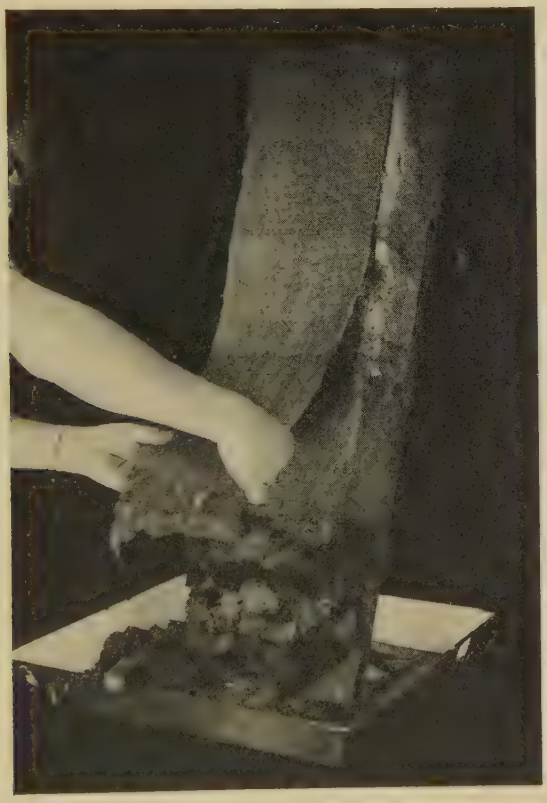

Fig. 5. The soil film is removed from the soil sample.

If the monolith is obtained by means of the iron frame, it can be treated with cellulose in a similar way. The soil in the frame is smoothed flush with the cutting edges of the frame and then the solution of cellulose is poured over the profile. In some cases several profiles can be preserved from one monolith. This can be done by first loosening the board at the back of the frame. A strip of hard-

board fitting exactly in the frame is then passed into the space between the board and the soil. By fixing the original board again with the screws the soil is pushed upwards into the frame to the thickness of the hardboard.

From the above it will be clear that in this case another sample must be taken by means of a pin-board for the preservation of the roots.

It is convenient to fix the profile film upon a background of hardboard or plywood next to the dried root system. The profile is fixed upon the board with the same cellulose solution, as is used for its preservation, in such a way that the upper side of this film is stuck onto the board. Later on a diluted solution of cellulose is sprayed with a vaporizer upon the front of the film in order to fix the soil particles.

For the fixation of the plastic sheet bearing the roots on the board we use a kind of glue, called Saba 810E. Other kinds of glue may answer quite as well, provided they are not dissolved in a liquid which affects the plastic sheet. Acetone or ketones are not suitable since they cause an irreversible wrinkling of the plastic. Moreover the glue must dry colourless.

The glue is painted upon the board, the plastic sheet with the roots is pressed on the board and finally the roots are stuck on the plastic sheet with the same glue. This glue is mixed then with a small amount of spreading agent since it is applied by means of a vaporizer.

It may be useful to frame the board but it is not strictly necessary. A picture of a complete set is given in Plate I.

The method as given above can be used without difficulty for sandy and light loam soils. It is not applicable to soils having a minute pore volume *i.e.* heavy, sticky clay soils, nor to soils having their pores permanently saturated with water, *i.e.* peaty soils. In these cases the cellulose solution cannot penetrate quickly enough. It appeared, however, that freezing of these profiles in a refrigerator might be helpful. In the frozen state the profile is first moistened by a highly diluted solution of cellulose, after which ample time is given for drying, before an undiluted solution of cellulose is added. As the preserved soil films removed from clay soils are generally thicker than those obtained from sandy soils, it is important to reinforce the cellulose layer by covering the monolith with strips of cloth before the cellulose solution has quite dried. In this manner the cloth is soaked with cellulose, and contributes afterwards to the

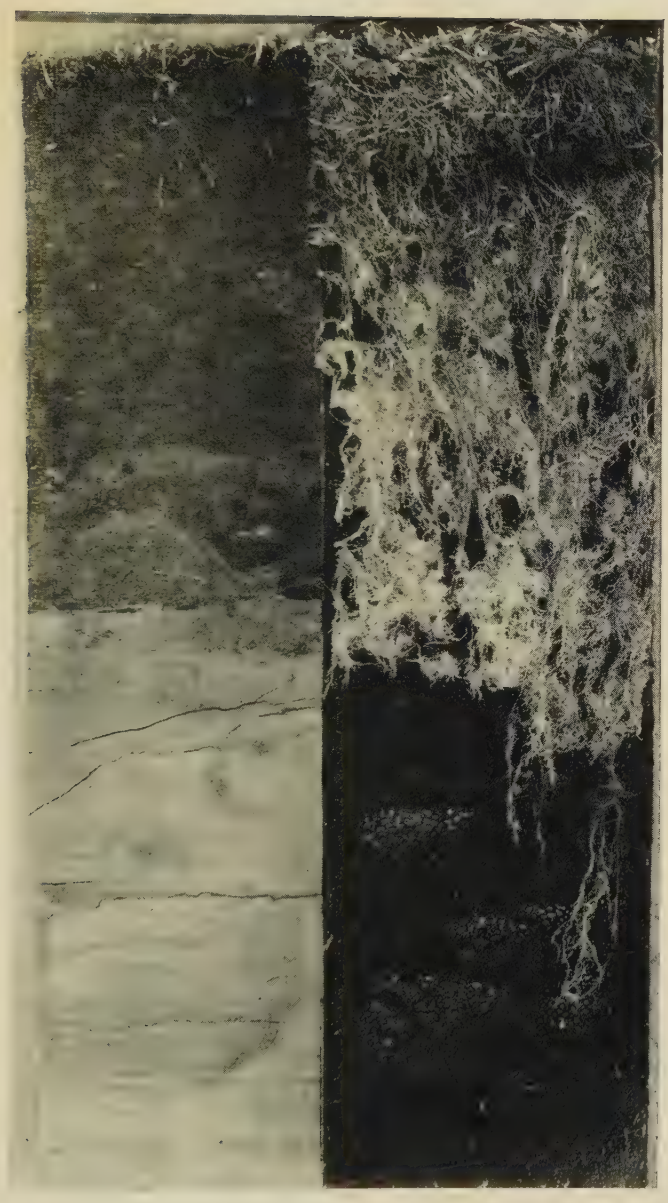

Plate I. Root development of grass on a sandy soil with a thin humous layer.

Plate II. Root development of grass on a sticky clay soil.

strength of the preserved film. When the cellulose solution is dried, the monolith is removed from the refrigerator.

These films cannot be removed from the monolith as easily as the sandy ones. It will generally be necessary to cut them free from the monolith when the latter has thawed. Afterwards the appearance of the structure can be improved by means of a pointed knife or pin. A fine cracking usually occurs in these profiles.

It is not possible to obtain the root systems from the heavy clay profiles in the usual way. The removal of this kind of soil by simple washing is either impossible or, in the most favourable cases, can only be performed by the sacrifice of much time.

This difficulty can be avoided by thoroughly drying the whole monolith at a temperature of $\pm$ 105°C and then soaking it in a solution of sodium pyrophosphate. We use solutions of 134 grams sodium pyrophosphate in 50 liters of water. Owing to the action of this solution the soil is usually rapidly peptised. After a couple of hours the soil can be washed easily and the roots can be glued upon board in the usual way (Plate II).

SUMMARY

Several investigators have published methods for the preservation of soil profiles by treating them with definite organic compounds.

In these no data are recorded as to the development of the crop roots in the preserved profiles.

In this article a modified technique is described for preserving soil profiles and their root systems simultaneously.

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PREFERENTIAL UTILIZATION OF AMMONIUM OVER NITRATE BY MICRO-ORGANISMS IN THE DECOMPOSITION OF OAT STRAW

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Ammonium and nitrate nitrogen, notwithstanding their divergent properties, generally have been considered about equally available as sources of nitrogen for micro-organisms (Richards and Norman [6])). Both ions are known to be utilized by large numbers of micro-organisms and both are commonly employed in microbiological experiments and used in the preparation of biological media. That one form may be more readily absorbed by micro-organisms than the other, however, was first suggested by Richards and Shrikhande [7]). They found that ammonium was absorbed more rapidly than nitrate, particularly during the early stages of attack in the decomposition of cereal straw. Earlier work by Niklewski [3]), in which straw was found to have an unfavorable influence on the utilization by crops of ammonium sulfate but none on the utilization of sodium nitrate, can be explained on the basis of preferential utilization of ammonium by the soil microflora.

In a study in our laboratory of the magnitude of biological interchange of nitrogen between the organic and inorganic phases in soil, evidence was obtained that ammonium was more available than nitrate. Reported herein are the results of a series of experiments where the forms of nitrogen are compared in utilization by a mixed microflora in the decomposition of oat straw.

*) Journal Paper J–2461, Project 1070 of the Iowa Agricultural Experiment Station. This work was supported in part by the Royal Agricultural College, Uppsala 7, Sweden, and was performed while the senior author was an Exchange Visitor at Iowa State College.

EXPERIMENTAL

Comparative utilization of ammonium and nitrate nitrogen was studied by applying ammonium nitrate in excess of the "nitrogen factor" to oat straw samples and then following the immobilization of each ion in subsequent periodical analyses by tracer techniques. To each of 3-g samples of air-dry oat straw was added 1.6% or 48 mg of nitrogen as ammonium nitrate. In one series of samples the ammonium ion was enriched with N^{15} and in a second series the nitrate ion was enriched. In both series the samples were wetted to 2/3 of the maximal water holding capacity and incubated at 26 ± 1°C. Samples were removed for analyses after 0, 5, 10, 20, 40 and 60 days of incubation.

The incubation apparatus was similar to that described by Bartholomew and Broadbent [1]. A continuous stream of moisture-saturated air, which had been freed of ammonia and carbon dioxide, was passed over the samples. The effluent air was passed through standard acid to absorb volatilized ammonia, and through standard alkali to absorb evolved carbon dioxide. The air streams were regulated in flow to about 0.4 l per hour.

The oat straw was mature and its dry matter content was 88.7%. On a dry weight basis the ash content was 7.3% and the nitrogen 0.49%. The straw was ground in a Wiley mill to pass a 2-mm screen.

At the conclusion of the decomposition periods, inorganic nitrogen was extracted by the method of Olsen [5] and nitrogen analyses were made of the organic, the ammonium and the oxidized forms of nitrogen. By analysis of N^{15} each of these nitrogen fractions was subdivided as to origin. *)

The results of the ammonium and nitrate utilization study are given in Table I. In a period of 10 days, 15 mg of ammonium nitrogen had been immobilized in contrast to only 1 mg of nitrate nitrogen. After 60 days the immobilization of ammonium and nitrate were respectively 17.8 and 2.0 mg/3 g of straw.

In another series of experiments the use of nitrate nitrogen was followed in soil treated with varying rates of corn stalk residue. In order to measure the rates of immobilization of soil and fertilizer nitrogen and of the mineralization of organic nitrogen an ex-

*) The N^{15} determinations were made through the courtesy of Dr H. Svec of the Institute for Atomic Research.

periment was conducted in which plant nitrogen was tagged in one instance and fertilizer nitrogen in another.

In one set of samples of Monona silt loam, corn stalks containing 1.3% of nitrogen enriched with N^{15} was added in 0.5 and 1.0 g amounts to 100 g soil. Nitrogen in the form of non-tagged calcium nitrate also was added at the rate of 5 mg N/100 g soil.

TABLE I

Biological utilization of ammonium and nitrate nitrogen in the decomposition of oat straw. Mg N/3g original straw after incubation

Nitrogen fraction	Origin of nitrogen	Incubation time in days					
		0	5	10	20	40	60
Organic	Straw	11.7	10.7	10.7	9.6	10.4	9.3
	NH_4–N	0.0	10.0	15.0	14.9	16.8	17.8
	NO_3–N	0.0	0.2	1.0	1.7	1.7	2.0
	Total	11.7	20.9	26.7	26.1	28.9	29.1
Ammonium	Straw	0.0	0.4	1.6	2.5	3.7	3.4
	NH_4–N	24.1	15.1	10.4	9.5	7.3	6.5
	NO_3–N	0.0	1.3	1.7	1.5	1.4	1.3
	Total	24.1	16.8	13.7	13.5	11.4	11.2
Nitrate	Straw	0.0	1.6	0.0	1.2	1.2	0.6
	NH_4–N	0.0	0.1	0.1	0.2	0.1	0.4
	NO_3–N	24.7	21.6	22.4	20.1	18.3	18.7
	Total	24.7	23.3	22.5	21.5	19.6	19.7

In a corresponding set of samples tagged calcium nitrate was used as the fertilizer and corn stalks containing 1.3% of non-tagged nitrogen served as the plant addition.

As a check on the effect of the plant material, one set of samples received only an addition of tagged calcium nitrate at the rate of 5 mg N/100 g soil and one set of samples received a 1% addition of non-tagged corn stalks plus 10 mg of nitrogen as tagged calcium nitrate.

At the conclusion of the several incubation periods samples were removed and the soluble nitrogen extracted with a *N* KCl solution made acid to pH 1 with HCl (Olsen[5]). By comparison of the inorganic and tagged nitrogen changes in the several series of samples the changes in the individual nitrogen sources were followed. The results are summarized in Table II.

Nitrate nitrogen was used by the soil flora during the first 10 days of incubation when microbiological activity and the demand for

nitrogen were high. During the 10–25 day interval, in the cultures containing corn stalks a small part of the immobilized nitrate nitrogen was mineralized. During the remainder of the incubation period little or no change occurred in the content of the tagged NO_3–N. Where no corn residues were added no significant changes occurred in the tagged-nitrate content of the soil. The data indicate that during the periods of high immobilization of nitrogen by micro-organisms and when ammonium was likely to be low, nitrate nitrogen was extensively absorbed, but, that when ammonium was being liberated more rapidly than nitrogen was being absorbed, nitrate nitrogen was utilized only to a minor extent.

TABLE II

Changes in soluble mineral nitrogen (mg/100 g soil) in a Monona soil following the addition of corn stalks residue and calcium nitrate fertilizer

Origin of soluble nitrogen	Sampling period in days				
	0	10	25	45	70
0.5 g corn stalks plus 5 mg N/100 g soil					
Plant	0.7	0.6	1.2	1.8	1.7
Fertilizer	4.4	2.1	3.0	2.9	2.2
Soil	5.2	3.9	6.1	7.2	7.7
Total	10.3	6.6	10.3	11.9	11.6
1 g corn stalks plus 5 mg N/100 g soil					
Plant	1.1	0.5	1.7	2.9	3.9
Fertilizer	4.5	0.3	1.6	1.8	1.5
Soil	5.7	2.7	5.5	7.5	7.9
Total	11.3	3.5	8.8	12.2	13.3
1 g corn stalks plus 10 mg N/100 g soil					
Fertilizer	9.4	1.4	4.1	3.8	4.1
Plant+soil	7.5	3.3	8.4	14.0	12.5
Total	16.9	4.7	12.5	17.8	16.6
5 mg N/100 g soil					
Fertilizer	4.5	4.4	4.5	4.7	4.8
Soil	5.5	6.6	6.6	7.7	8.3
Total	10.0	11.0	11.1	12.4	13.1

The observations of preferential immobilization of ammonium were followed by an investigation of the influence of ammonium and nitrate salts on the rate of decomposition of oat straw. Straw samples were prepared and incubated as previously described. One set received no nitrogen, a second set ammonium nitrate, a third set ammonium sulfate and a fourth set sodium nitrate. The results are shown in Table III.

TABLE III

Nitrogen immobilization and carbon dioxide production in the decomposition of oat straw when various nitrogen salts are added

Analysis/3 g original straw after 10 days incubation	Nitrogen treatment			
	No N	48 mg N NH_4NO_3	24 mg N $(NH_4)_2SO_4$	24 mg N $NaNO_3$
Organic N, mg	11.5	26.8	26.1	19.8
Net change in inorganic N, mg	0.0	−14.1	−14.1	−8.1
Carbon dioxide production, mg	294	689	674	452
pH conclusion of experiment	7.7	5.8	4.6	8.1

Nitrogen immobilization and carbon dioxide production were both markedly higher where ammonium was supplied then where nitrate was added to the straw. However, where ammonium salts were supplied, the systems became rather acid in comparison to the alkaline reaction produced where sodium nitrate was added.

To study the influence of nitrogen source at constant pH a series of oat straw samples were prepared and incubated as previously described. In one set the pH was adjusted to 7.5 with phosphate buffer. In a second set the pH was controlled near 6.0. Both sets were incubated for 10 days. The results are shown in Table IV.

TABLE IV

Influence of reaction on immobilization of nitrate and ammonium nitrogen in the decomposition of oat straw

Analysis/3 g original straw after 10 days incubation	Treatments			
	Acid systems		Alkaline systems	
	24 mg N as $NaNO_3$	24 mg N as $(NH_4)_2SO_4$	24 mg N as $NaNO_3$	24 mg N as $(NH_4)_2SO_4$
Organic N, mg	18.0	26.8	15.3	29.2
Net change in inorganic N, mg	−6.9	−14.5	−4.4	−17.5
Carbon dioxide production, mg	408	519	330	575
pH range	6.0–6.1	6.0–5.5	7.5–7.8	7.5–6.7

Nitrogen immobilization as well as carbon dioxide evolution shows that the nitrogen source was the major factor in modifying the rate of decomposition of the oat straw, and the reaction of the systems appeared to have had only a minor influence. Nitrate appears to have been most effectively used in acid and ammonium in alkaline systems.

The appearance of the samples at the conclusion of incubation suggested that perhaps marked differences in the microfloras had been induced by the reaction. On the acid samples was a heavy

growth of fungi while on the alkaline samples no fungus colonies were detected. Plate counts on rose-bengal, dextrose-nitrate agar and on soil extract agar confirmed the high incidence of fungi in the acid systems and the marked predominance of bacteria in the alkaline systems. Both groups of organisms, however, were able to effect a more rapid decomposition of oat straw when supplied with ammonium sulfate than when supplied with sodium nitrate.

In the previous decomposition studies the moisture contents were adjusted to 2 3 of the maximal water-holding capacity or about 330% of the dry weight of the straw. Since moisture content can modify the composition of the microflora as well as the rate of decomposition an experiment was conducted where decomposition was permitted at a series of moisture levels. The straw samples were prepared as previously described and the incubations were continued for 10 days. The results are shown in Table V.

TABLE V

Influence of moisture content on the utilization of ammonium and nitrate nitrogen in the decomposition of oat straw

Nitrogen addition	Analysis/3 g original straw after 10 days incubation	Moisture contents % of dry wt. of straw					
		80	160	240	320	400	480
24 mg N as $NaNO_3$	Organic N, mg	21.8	18.2	17.1	17.3	16.0	14.3
	Net change in org. N, mg	+10.3	+ 6.7	+ 5.6	+ 5.8	+ 4.5	+ 2.8
	Net change in inorg. N, mg	— 9.6	— 6.6	— 4.5	— 6.1	— 5.6	—17.5
	Carbon dioxide evolved, mg.	447	399	373	378	365	320
	pH conclusion of expt.	8.4	8.0	8.1	8.0	8.2	8.4
48 mg N as NH_4NO_3	Organic N, mg	—	27.0	—	27.8	—	21.4
	Net change in org. N, mg	—	+15.5	—	+16.3	—	+ 9.9
	Net change in NO_3–N, mg	—	— 4.7	—	— 2.4	—	— 9.7
	Net change in NH_4–N, mg	—	— 9.8	—	—13.7	—	— 7.4
	Carbon dioxide evolved, mg.	—	653	—	537	—	409
	pH conclusion of expt.	—	6.2	—	6.5	—	7.4

Where ammonium nitrogen was available, nitrogen immobilization and carbon dioxide evolution were considerably higher at all moisture levels than were only nitrate nitrogen was supplied. Moisture content, within the range studied, had no differential influence upon utilization of ammonium and nitrate. At saturation, decomposition with both ammonium and nitrate was retarded and some nitrogen losses occurred, presumably by denitrification. The data suggest that nitrate may be more readily utilized at low than at high moisture contents.

Since the nitrogen in many organic substances is easily ammonified, it would be expected that the addition of such a source of nitrogen to oat straw would tend to supply ammonium to the microflora with the result that added nitrate would be unused. To test this hypothesis the experiment reported in Table VI was conducted. Peptone was used as the source of organic nitrogen and was compared with sucrose as a non-nitrogenous, readily available material. Part of the samples were kept at slightly acid reaction with phosphate buffer and another set kept slightly alkaline. To all samples was added 24 mg nitrogen as sodium nitrate.

TABLE VI

Influence of peptone and sucrose on the utilization of nitrate nitrogen in the decomposition of oat straw

Analysis/3 g original straw after 10 days incubation	Acid systems			Alkaline systems		
	No carbon addition	50 mg carbon added as		No carbon addition	50 mg carbon added as	
		Peptone	Sucrose		Peptone	Sucrose
Organic N, mg	18.0	29.0	23.7	15.6	22.4	17.5
Net change org. N, mg	+ 6.5	+ 3.3	+12.2	+ 4.1	+ 3.3	+ 6.0
Net change NO_3–N, mg	— 6.9	— 3.8	—12.6	— 4.1	— 1.6	— 7.6
Net change NH_4–N, mg	—	—	—	—	+ 4.9	—
CO_2 evolved, mg	408	650	569	273	416	414
pH range	6.0–6.1	6.0–6.1	6.0–6.5	7.2–7.6	7.2–7.4	7.2–7.7

Less nitrate nitrogen was immobilized where peptone was added to the samples than where no additions were made notwithstanding the total decomposition was as high or higher with peptone as with sucrose. Substantial amounts of nitrate were immobilized where sucrose was added.

DISCUSSION

Ammonium nitrogen not only was absorbed in greater quantities than nitrate by the microfloras involved in the decomposition of oat straw but also served to promote a more rapid decomposition. An explanation of this phenomenon is yet a matter of conjecture. Since ammonium was absorbed to the exclusion of nitrate when equal concentrations of both were present, it must be concluded that there is a difference between the two ions in the mechanism and ease of absorption and utilization. In view of the possible

reactions of the two ions in or with organic materials it is difficult to explain the relative utilization on the basis of availability in the substrate. The greater uptake of ammonium was observed under such a variety of nutrient and environmental conditions that it appears unlikely that associated ions could have had more than a minor influence on differential nitrogen absorption. The fundamental explanations must lie in the biochemical reactions involved in, or attendant to, the absorption of nitrogen by the micro-organism and the subsequent conversion to protein and other organic substances.

Although ammonium and nitrate nitrogen have generally been considered of about equal value as fertilizer materials under a wide range of conditions, there may be peculiar sets of circumstances where each would have advantages over the other. In laboratory and greenhouse studies a larger proportion of added nitrate nitrogen has been recovered from soil by both chemical extraction and plant uptake than has ammonium *) (Bartholomew and Hiltbold[2]), Norman and Krampitz[4]), Thornton[8])). The lower recovery of ammonium is presumed to result from fixation on or in the clay materials. Since the studies have been of short duration, 1 to 3 months, the carry-over effects of ammonium in comparison to nitrate have not been evaluated.

The present study indicates that ammonium should be superior to nitrate in the production of composts and artificial manures because it promotes more rapid initial decomposition and a greater immobilization of nitrogen in organic form. It is true that ammonium added to soil under conditions favorable in moisture and temperature will not long remain as ammonium. Nitrifying organisms will rapidly oxidize it to nitrate. However, plant residues which have not had close contact with the soil do not, in general, have an active nitrifying flora. As shown in Table I, when decomposition took place apart from soils, very little of the ammonium was oxidized to nitrate even at the end of a 60 day period of incubation.

Another factor to be considered is the rapidity of the biological response to the nitrogen source. The data indicate that maximum uptake of nitrogen had been closely approached in about 10 days

*) Jansson, S. L., Unpublished data.

under favorable environmental conditions. During this period of time, even in soil with an active nitrifying flora, sufficient ammonium should remain available to result in a more rapid initial decomposition and greater nitrogen absorption by the organisms in the decomposing residues than if nitrate were applied. Ammonium, therefore, should also be the better nitrogen source to apply when fertilizing to offset the deleterious influence on crop production of cereal straws, corn stalks and other low nitrogen crop residues.

SUMMARY

Ammonium was compared with nitrate as a source of nitrogen for micro-organisms in the decomposition of oat straw. When both ions were added in sufficient and equal quantities the organisms utilized the ammonium ion almost exclusively. The relative absorption of the two ions was determined by means of tagged nitrogen.

Ammonium was also found to promote a greater total immobilization of nitrogen and more rapid decomposition of the oat straw under a wide range of environmental conditions. The results suggest that ammonium may be preferable to nitrate in the production of composts and to have certain advantages when nitrogen is applied to promote decomposition of crop residues.

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CHARACTERIZATION OF SOIL PHOSPHORUS BY ANION EXCHANGE RESIN ADSORPTION AND P^{32}-EQUILIBRATION *)

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A fundamentally new approach to the problem of estimating soil-P availability was made possible by the advent of P^{32}. When a solution of orthophosphate labeled with P^{32} is added to soil, the added P mixes with the soil P in solution. At the same time, it begins to equilibrate with solid-phase soil P. Equilibration with organic P takes place as a result of biological synthesis and mineralization of P-bearing organic compounds. Equilibration with inorganic P takes place as a result of dissociation of phosphate groups from the site of binding in the soil, and their replacement from the solution phase by other phosphate groups, some of which contain P^{32}. Over a period of a few days, and perhaps for a much longer time, only a minor part of the equilibration is with the organic P. When equilibrium has been attained, the quantity of soil P with which the added P has equilibrated can be calculated from the quantity of P and the proportion of the P^{32} in the solution phase.

As a means of estimating soil-P availability, equilibration with P^{32} has two advantages. First, except for the greater quantity of water, conditions during measurement are not unnatural. The measurement may be made without addition of the acids, bases, or complexing or precipitating reagents commonly employed in chemical extraction procedures. Second, the measurement reflects the behavior of the soil P. It is not complicated by other characteristics of the soil,

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except as the latter influence the behavior of the P. Such is not the case with the usual chemical extractants. Equilibration with P^{32} has also two disadvantages. First, calculation of the quantity of soil P with which the added P has equilibrated is strictly valid only at equilibrium. Since the process continues indefinitely, the required condition of equilibrium does not prevail. Second, the extent of kinetic exchange of phosphate for phosphate may not be entirely satisfactory as an estimate of soil-P availability. Adsorption of phosphate by plants is largely a one-way process in which phosphate is removed from solution. Although electrical neutrality is maintained, the ion that replaces phosphate derived from the solid phase is usually not phosphate. Hydroxyl and silicate derived from the solution are probably the major replacing ions. Whether or not the phosphate for phosphate exchange measured by P^{32} properly characterizes soil P with respect to this other type of exchange is not certain.

As an alternative method for characterizing soil P, the use of an anion exchange resin was conceived. It appeared that if an anion exchange resin would remove P from the soil solution, its action would be more nearly analogous to that of the plant than the phosphate for phosphate exchange measured by P^{32}. The resin would remove the phosphate from solution, but would not return another phosphate ion in exchange. If the anion initially present on the resin were chloride, for example, the chloride exchanged would remain in solution, and some other anion such as hydroxyl or silicate would take the place of the phosphate removed from the solid phase. Strong-base type anion exchange resins remove anions from solution over a wide pH range, can be added in neutral form, and are only slightly buffered over the pH range of interest in soils. Their use might thus embrace both the advantages mentioned above for P^{32}-measurements without the disadvantages. The purpose of the work reported in the present paper was to investigate the use of an anion exchange resin for soil-P characterization.

METHODS

The method used for determining the apparent quantity of soil P that equilibrates with P^{32} was essentially that of McAuliffe *et al.* [6]). Five-gram samples of 100-mesh soil in an Erlenmeyer flask were agitated continuously with 25 ml of water for 3 to 4 days on a wrist-action shaker.

A 1-ml aliquot of P^{32}-solution was then added, and agitation was continued. The P-concentration of the P^{32}-solution was similar to that of inorganic P in the solution in contact with the soil. At the end of the reaction period, samples of the solution were obtained for analysis by centrifugation.

The P^{32} used was obtained in the form of a dilute solution of H_3PO_4 containing 0.025 mg of P^{31} per millicurie of P^{32}. The P^{32} was free from radioactive impurities, as indicated by half-life measurements. Molybdiphosphate precipitation [5]) after heating with liquid Br_2 indicated that 99.8% of the P^{32} was in orthophosphate form. Radioassays were made using a solution counter. The quantities of P^{32} employed were adjusted so that about 40 counts per second were obtained; about 10,000 counts were recorded in each case.

For P-adsorption measurements, the Cl-form of the strong-base anion exchange resin Dowex-2 was used throughout. For use, the resin was air dried; particles passing through a 32-mesh sieve were discarded. One-gram samples of resin were used in all experiments.

In experiments with solutions, the analytical procedure involved digesting the resin with H_2SO_4, with subsequent additions of P-free H_2O_2, followed by $HClO_4$. (The P contained in the peroxide was removed by adsorption on several successive batches of resin.) P was determined by the Dickman and Bray [3]) method.

In all experiments with soil, 100-mesh soil was used. One gram each of resin and soil, and 100 ml of water, were used throughout, except in a resin-soil ratio experiment, where the weight of soil was varied. The suspensions were shaken continuously on a wrist-action shaker, except in an experiment on method of shaking. After the adsorption period, the resin was separated from the soil by washing the suspension through 82-mesh bolting cloth mounted on a Büchner funnel. P adsorbed by the resin was exchanged by digesting the resin with 25 ml of a 10% solution of NaCl on a steam plate for 45 minutes, followed by leaching with additional portions of NaCl solution until 100 ml of filtrate were obtained. P extracted was determined by the Dickman and Bray [3]) method. The extraction procedure gave 98% recovery of adsorbed P.

RESULTS AND DISCUSSION

Investigation of technique

Several experiments were conducted to find whether conditions could be adjusted in such a way that adsorption measurements made using soils would reflect the behavior of soil P rather than that of the resin or the accessory characteristics of the soil.

The first experiment was conducted to determine the range over which adsorption is quantitative. When 0.2, 1, 5, and 10 mg of P as KH_2PO_4 in 200 ml of water were shaken overnight with 1 g of resin,

adsorption amounted to 0.2, 1.0, 4.9, and 8.0 mg of P with the respective additions. In the work to follow with soils, the quantities of P to be dealt with are of the order of 0.1 mg or less. The range encountered in soils is evidently well within the capacity of the resin for quantitative adsorption.

The second experiment was designed to find whether the rate-controlling process in adsorption is diffusion of phosphate inside or outside the resin particles when the resin is agitated in the solution. The rate of adsorption was investigated by placing the resin in a bolting-cloth sack fitted over the end of a glass stirrer shaped like an inverted Y, and lowering the rotating stirrer for the desired length of time into 12 liters of solution containing 1 ppm of P as KH_2PO_4. On raising the stirrer from the solution, the resin was washed immediately with a stream of water. The quantities of P adsorbed in this way were small enough to justify the assumption that the concentration of P in the bulk of the solution remained constant throughout. Under these conditions, the equations of B o y d, A d a m s o n, and M y e r s [1] may be used to distinguish between the two limiting conditions. If diffusion in the resin particles is rate-determining, the results will be described by the equation

$$\frac{Q_t}{Q_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \left(\frac{1}{n^2} \cdot e^{-D\pi^2 n^2 t/r^2} \right) \qquad (1)$$

On the other hand, if the rate of adsorption is determined by the rate of diffusion through a boundary film of liquid or by the actual exchange, the results will be described by the equation

$$-\log\left(1 - \frac{Q_t}{Q_\infty}\right) = kt \qquad (2)$$

In these equations, Q_t refers to the quantity of P adsorbed at time t, Q_∞ is the quantity adsorbed at equilibrium, D is the diffusion coefficient of P in the particles, r is the particle radius, and k is a constant.

B o y d and co-workers pointed out that to determine whether experimental data are represented by equation (1), a value of Bt is found for each observed value of Q_t/Q_∞. B is equal to $D\pi^2/r^2$. The proper value of Bt is found by graphical interpolation in a table of values they give. When values of Bt obtained in this way are divided by t, the resulting values of B should be constant. Use of

this technique with the experimental data showed that B increased with t, which indicates that diffusion of P within the resin particles did not determine the rate of P-adsorption.

If the experimental data can be described by equation (2), a plot of $-\log (1 - Q_t / Q_\infty)$ against t will yield a straight line. Figure 1 shows that the experimental data can be represented by this equation. The results indicate, therefore, that the rate of P-ad-

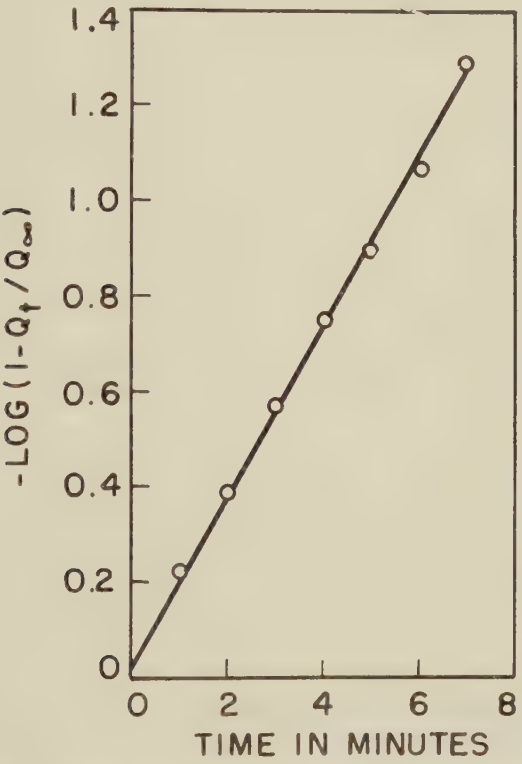

Fig. 1. Test of boundary-film diffusion hypothesis.

sorption from dilute solutions such as those in soils is controlled by the rate of diffusion through a thin film of solution surrounding the resin particles or by the actual rate of ion exchange. If such is the case, the rate of adsorption will be proportional to the concentration gradient across the surface film or to the concentration in solution. This prediction was verified in a third experiment, conducted in a manner similar to the second. Results are shown in Figure 2.

Although neither of the foregoing tests permits one to distinguish between the film-diffusion and mass-action mechanisms, the probability is small that the rate of ion exchange was the limiting process. Experiments with systems similar to the one used in the present investigation show that up to a point the rate of adsorption increases with the rate of stirring. Rate of stirring would be without effect if the rate of exchange were limiting.

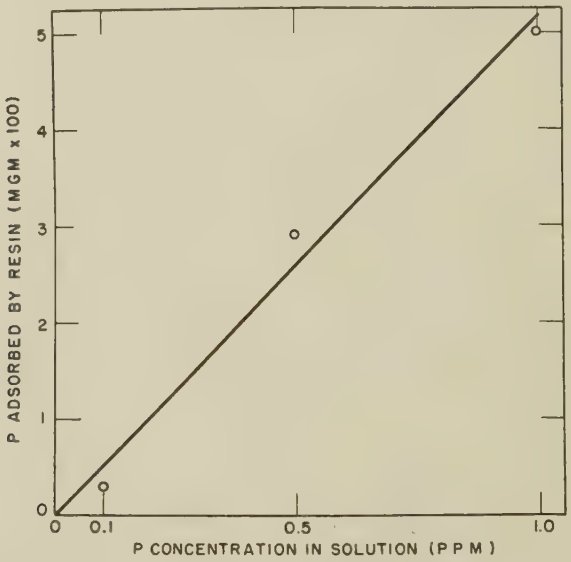

Fig. 2. Phosphorus absorbed by resin in one minute *vs.* concentration of solution.

The fourth experiment was conducted to investigate the effect of soluble salts on the rate of adsorption of P by the resin. Soluble salts provide competing anions that increase the concentration of P in solution at equilibrium, and hence reduce the concentration gradient across the film of solution surrounding the resin particles. Four-liter quantities of solutions were prepared to contain 0.125 ppm of P as KH_2PO_4, together with various concentrations of NaCl up to 200 ppm. The solutions had a reaction of about pH 6.7. The bolting-cloth sack containing 1 g of resin was agitated by an electric stirrer for 6 minutes in water, for 3 minutes in the phosphate-NaCl solution, and then again in water for 15 seconds. The P was

extracted and determined as before. The results of the experiment may be expressed by the equation

$$\hat{y} = 35 - 0.024x \qquad (3)$$

where $\hat{y}$ is the estimated adsorption of P as γ per g of resin in 3 minutes, and x is the concentration of NaCl in ppm. The regression coefficient is significant at the 5% level. According to equation (3), a concentration of 20 ppm of NaCl is sufficient to reduce P-adsorption by about 1%. The salt content of arable soils seldom exceeds 2000 ppm, which would yield a concentration of about 20 ppm when 1 g of soil is suspended in 100 ml of water, as in the work with soils to be described below. These results indicate that the effect of soluble salts on adsorption of P from soil by the resin is usually of minor importance.

The purpose of the fifth experiment was to find the effect of pH on adsorption of P by the resin. Four-liter quantities of solutions were prepared, all containing 4.3 ppm of Cl as HCl and 0.125 ppm of P as KH_2PO_4. The pH was varied by changing the addition of NaOH. The adsorption technique was the same as that described for the preceding experiment. The results are given in Table I.

TABLE I

Adsorption of P by resin from solutions at different pH values				
Initial pH of solution	4.0	5.5	6.8	8.7
Final pH of solution	4.1	5.5	6.8	8.5
P adsorbed per g of resin, γ	35	30	29	29

Analysis of variance showed that differences in P-adsorption between pH values were significant at the 1% level. Most of the effect of pH occurred in the range between pH 4 and pH 5.5, where adsorption decreased about 14%. The differences in adsorption observed between pH 5.5, 6.8, and 8.5 were within experimental error.

Supplementary work was done on the pH effect in an attempt to determine whether the cause was a change in the rate of diffusion of P across the film of solution surrounding the resin particles or a change in concentration of P at the surface of the resin particles. The best experimental test of the latter appeared to be the concentration of P in solution at different pH values after solutions of constant quantity and P-concentration but differing in pH value

had been equilibrated with a constant quantity of resin. The reason for applying this test is that one of the assumptions underlying equation (*2*) requires the concentration of P at the surface of the resin during adsorption to be the equilibrium concentration for the particular quantity of P adsorbed. The adsorption behavior was shown above to be accounted for by equation (*2*), which indicates the correctness of the assumption. At equilibrium, the concentration gradient causing diffusion toward the resin is zero. The concentration of phosphate in solution at equilibrium at different pH values can thus be used as a criterion for ascertaining whether the effect of pH on the rate of adsorption can be accounted for by a higher concentration at the resin surface at some pH values than at others. Accordingly, 500-ml quantities of solution containing 0.1 ppm of P as KH_2PO_4 and different concentrations of HCl or NaOH were equilibrated for 3 or 4 days with 1 g of resin. The pH values of the equilibrium solutions ranged between 4.6 and 7.4. Irrespective of the pH, however, the P-concentration was essentially zero. These results indicate that variations in concentration of P at the surface of the resin particles during adsorption were not responsible for the pH effect, which leaves the effect of pH on the diffusion coefficient as the alternative possibility. Attempts to find whether or not the change in diffusion coefficient of P with pH would account for the results were unsuccessful because of technical difficulties. In the absence of information to the contrary, therefore, the variation in rate of adsorption with pH may be attributed to the effect of pH on the diffusion coefficient of the P. The pH effect thus appears to result from an inherent characteristic of the P and not of the resin.

The sixth experiment was designed to determine the ratio of resin to soil required to produce the maximum rate of P-removal from the soil. For this purpose, 1-g samples of resin were shaken continuously with 0.5, 1, 2, 3 and 5-g samples of soil in 100 ml of water. At the end of 2 hours, the resin was separated from the soil and analyzed for P. The results in Table II indicate that the rate of adsorption was independent of the ratio of resin to soil at a value of 1 to 1 or greater. In other words, with a ratio of 1 to 1 or greater, the quantity of P adsorbed by the resin is controlled by the rate of release of P from the soil, and not by the rate of adsorption. This behavior can be accounted for on the basis that with a 1 to 1 ratio

enough resin is present to maintain the concentration of P in solution at an extremely low value. Tests made on solutions obtained from Fargo clay, Waukegan silt loam, and Langdon loam showed that after shaking 1 g each of resin and soil with 100 ml of water for 2 hours, the inorganic P concentrations were 0.009, 0.012, and 0.015 ppm, respectively. These values are probably overestimates, because of release of P by fine soil fractions during centrifuging after the resin particles had been centrifuged to the bottom of the tube. In a supplementary experiment with several soils, the rate and method of continuous shaking were found to have no consistent effect on the quantity of P adsorbed by the resin.

TABLE II

Phosphorus adsorbed by resin from soils in two hours with different ratios of resin to soil

Ratio of resin to soil by weight	P adsorbed by resin in 2 hours, ppm of soil	
	Langdon loam	Fargo clay
0.2	10.4	20.8
0.33	10.6	22.6
0.5	10.9	23.6
1.0	14.1	28.3
2.0	13.8	28.6

The results of the foregoing experiments indicate that when 1 g of resin is shaken continuously with 1 g of soil in 100 ml of water, the resin acts as a constant sink for the P in solution. This behavior may be attributed to the relatively large excess of resin over the total P in solution and to the rapidity of diffusion of adsorbed P in the resin. The rate of P-adsorption by the resin is proportional to the P-concentration of the bulk of the solution, which in turn is proportional to the rate at which P appears in solution from the soil solid phase. The resin maintains the concentration of P in solution at such a low value that the quantity of P adsorbed by the resin and the rate of P-adsorption by the resin may be taken, respectively, as estimates of the quantity of P released and the rate of P-release from the soil. The technique apparently yields a measurement of the release of P that is independent of the accessory characteristics of the soil, except as the latter may influence the release of P.

Rate curves with P^{32} equilibration and resin adsorption

Table III shows the comparative amounts of P in different soils equilibrated with P^{32} and adsorbed by an exchange resin after different lengths of time. Although the values obtained by the two methods are definitely different, the relatively close agreement between the two suggests that much the same P is involved. The coincidence, however, is fortuitous. Other experiments showed that equilibration of soil samples with water prior to resin adsorption in the manner used in the P^{32}-experiments considerably reduced the amount of P adsorbed by the resin. Presumably, the dehydration resulting from air-drying inactivates part of the adsorption sites. Rehydration on wetting gradually increases the adsorption capacity, thereby reducing the rate of P-release to the resin.

With both techniques, the amount of soil P involved increased rapidly at first, and then more slowly. The shape of the curves is presumably a function of three fundamental properties of the various phosphate groups of soils, namely, quantity, degree of dissociation, and accessibility to the solution phase. Fig. 3 shows the amount of P adsorbed from Waukegan silt loam after different lengths of time. The shape of the curve for P adsorbed during the entire 72-hour period indicates the existence of two simultaneous reactions differing in rate, one largely completed in the first 2 or 3 hours, and the other still continuing at the end of 72 hours. The shape of the inset curve for P adsorbed during the first 2 hours indicates the existence of still another and more rapid reaction. Apparently, therefore, there were three simultaneous reactions differing in rate. Thus, if P_r is the amount of P adsorbed by the resin in time t,

$$P_r = P_{r1} + P_{r2} + P_{r3}$$

where P_{r1}, P_{r2}, and P_{r3} represent the P adsorbed as a result of the three different reactions. If the rate of adsorption of P by the resin in each reaction is proportional to the quantity of unadsorbed P of that form remaining in the soil,

$$dP_{r1}/dt = k_1' P_{s1} \qquad (4a)$$

$$dP_{r2}/dt = k_2' P_{s2} \qquad (4b)$$

$$dP_{r3}/dt = k_3' P_{s3} \qquad (4c)$$

where P_{s1}, P_{s2}, and P_{s3} are the quantities of unadsorbed soil P that

TABLE III

Soil phosphorus adsorbed by resin and equilibrated with P^{32} after different lengths of time

Time, hours	Soil phosphorus, ppm of soil									
	Fargo clay				Waukegan silt loam				"Marshall-like" silt loam	Langdon loam
	Equilibrated with P^{32}			Adsorbed by resin	Equilibrated with P^{32}			Adsorbed by resin		
	Solid-phase P	Solution-phase P	Total		Solid-phase P	Solution-phase P	Total		Adsorbed by resin	Adsorbed by resin
0.25	18.3	2.0	20.3	14.0	26.1	9.1	35.2	25.0	5.0	7.9
0 5	19.5	1.1	20.6	18.4	31.3	7.4	38.7	31.0	6.2	9.9
1	24.2	2.0	26.2	23.6	36.4	9.5	45.9	38.7	7.4	12.8
2	29.1	2.4	31.5	28.3	43.5	9.5	53.0	46.5	8.6	14.1
4	35.2	2.4	37.6	30.5	50.3	9.6	59.9	52.8	9.2	14.9
8	36.4	1.8	38.2	36.6	54.8	8.8	63.6	59.2	9.6	16.6
12	38.4	1.9	40.3	38.4	58.9	8.8	67.7	63.3	9.3	17.1
24	44.6	1.9	46.5	43.6	69.7	8.7	78.4	73.6	9.9	19.6
72	—	—	—	50.7	—	—	—	90.7	11.5	22.8

contribute to the respective reactions at the time t, and k_1', k_2', and k_3' are constants. On the basis of these assumptions, the overall curve was resolved into three component curves using the approach described below.

If equations (*4a*), (*4b*) and (*4c*) are integrated, they have the form

$$P_{r1} = C_1(1 - e^{-k'1t}) \qquad (5a)$$

or

$$P_{r1} = C_1(1 - 10^{-k_1 t}) \qquad (5b)$$

if the base e is changed to the base 10. The parameter C_1 represents the initial quantity of P_{s1} present in the soil. Analogous equations may be written for P_{r2} and P_{r3}. The overall equation representing the sum of the three reactions is thus

$$P_r = P_{r1} + P_{r2} + P_{r3} =$$
$$= (C_1 + C_2 + C_3) - (C_1 \times 10^{-k_1 t} + C_2 \times 10^{-k_2 t} + C_3 \times 10^{-k_3 t}) \quad (6a)$$

which may also be written

$$\log (C_1 + C_2 + C_3 - P_r) =$$
$$= \log (C_1 \times 10^{-k_1 t} + C_2 \times 10^{-k_2 t} + C_3 \times 10^{-k_3 t}) \quad (6b)$$

If the time of adsorption has been long enough to permit the rapid and intermediate reactions to go essentially to completion, $C_1 \times 10^{-k_1 t}$ and $C_1 \times 10^{-k_2 t}$ will be small, and may be neglected. Equation (*6b*) then simplifies to

$$\log (C_1 + C_2 + C_3 - P_r) = \log C_3^{-k_3 t} \qquad (7)$$

from which it is evident that a plot of $\log (C_1 + C_2 + C_3 - P_r)$ against t will yield a straight line of slope k_3 and intercept $\log C_3$. To obtain the values of $\log C_3$ and k_3, a guess was first made of the value of $(C_1 + C_2 + C_3)$. The latter was obtained from a plot of P_r against t, and represents the value of P_r when t becomes infinite. Using the guessed value of $(C_1 + C_2 + C_3)$ and the observed values of P_r and t, a plot was made of $\log (C_1 + C_2 + C_3 - P)$ against t. The process was repeated with different guessed values of $(C_1 + C_2 + C_3)$ until a value (94.7) was found that yielded the best straight line. (The portion of the line calculated from low values of P_r and t was curved because of the rapid and intermediate reactions.) The straight line was found to have the slope $k_3 = 0.015$

and the intercept corresponding to $C_3 = 47.9$. The equation for the slow reaction is thus

$$P_{r3} = 47.9\ (1 - 10^{-0.015t}) \qquad (8)$$

By means of equation (*8*), a value of P_{r3} was calculated for each observed value of t and subtracted from the corresponding value of P_r to obtain a series of values of $(P_{r1} + P_{r2})$. The latter represent the amounts of P adsorbed as a result of the rapid and intermediate reactions only. Equation (*6b*) may then be simplified to

$$\log [(C_1 + C_2) - (P_{r1} + P_{r2})] = \log [C_1 \times 10^{-k_1 t} + C_2 \times 10^{-k_2 t}] \qquad (9)$$

If the time of adsorption has been long enough for the rapid reaction to become essentially completed, $C_1 \times 10^{-k_1 t}$ will be small, and may be neglected. Equation (*9*) may then be further simplified to

$$\log [(C_1 + C_2) - (P_{r1} \times P_{r2})] = \log C_2^{-k_2 t} \qquad (10)$$

Since the value of $(C_1 + C_2)$ was known from

$$C_1 + C_2 = (C_1 + C_2 + C_3) - C_3 = 94.7 - 47.9 = 46.8,$$

$\log C_2$ and k_2 were evaluated from a plot of $\log [(C_1 + C_2) - (P_{r1} + P_{r2})]$ against t. C_2 was found to be 28.2, and k_2 to be 0.45. The equation for the intermediate reaction is thus

$$P_{r2} = 28.2\ (1 - 10^{-0.45t}) \qquad (11)$$

By means of equation (*11*), a value of P_{r2} was calculated for each observed value of t and subtracted from the corresponding value of $P_{r1} + P_{r2}$ to obtain a series of values of P_{r1}. Equation (*6b*) may then be simplified to

$$\log (C_1 - P_{r1}) = \log C_1 - k_1 t \qquad (12)$$

Since the value of C_1 is known from

$$C_1 = (C_1 + C_2) - C_2 = 46.8 - 28.2 = 18.6,$$

k_1 is the only unknown in equation (*12*).

The rapid reaction was apparently completed within the first 15 minutes, because all calculated values of P_{r1} were equal to or greater than C_1 (the average value of P_{r1} was 18.9). Since no time intervals short enough to produce a positive value of $(C_1 - P_{r1})$ were employed, k_1 could not be evaluated by solving equation (*12*).

As a substitute, an arbitrary estimate of k_1 was obtained by making a free-hand curve of P_r against t. In this way, the value $P_r = 21$ was obtained for $t = 0.125$ hour. P_{r3} and P_{r2} were then evaluated for this same time interval from equations (*8*) and (*11*). The resulting estimate $P_{r1} = 17.4$ was substituted in equation (*12*) to obtain the value $k_1 = 9.5$. The equation for the rapid reaction is thus

$$P_{r1} = 18.6\,(1 - 10^{-9.5t}) \qquad (13)$$

By adding equations (*8*), (*11*), and (*13*), the equation for the total P adsorbed in all three reactions may be written in the form of equation (*6a*):

$$P_r = 94.7 - (18.6 \times 10^{-9.5t} + 28.2 \times 10^{-0.45t} + 47.9 \times 10^{-0.015t}) \qquad (14)$$

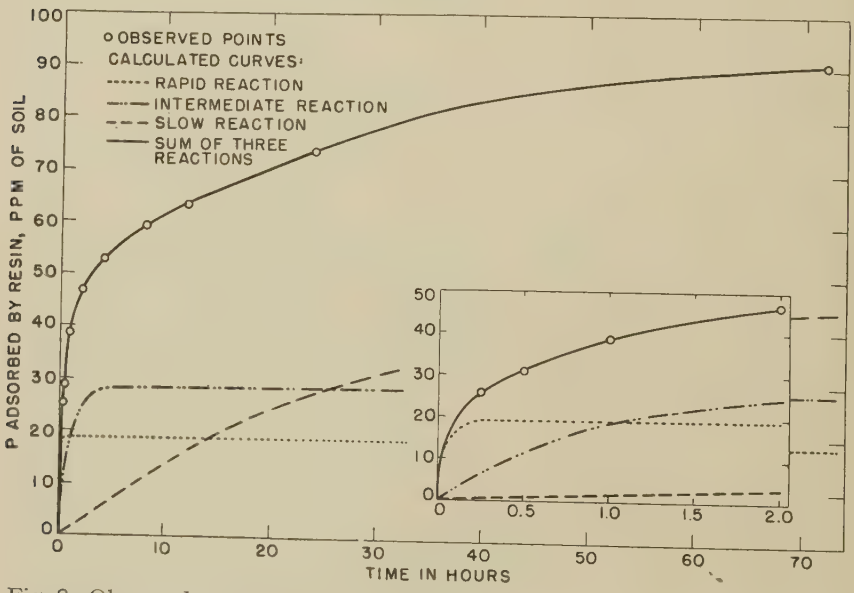

Fig. 3. Observed amounts of phosphorus absorbed by Dowex-2 from Waukegan silt loam after different lengths of time, and calculated curves representing different reactions involved.

The three calculated curves and their sum are shown together with the observed data in Fig. 3. Calculated and observed values agree the closely, which shows that the data are in agreement with the assumed existence of three simultaneous first-order reactions. No appreciable improvement in the fit would occur from assuming

more than three, but a definitely poorer fit would result from assuming fewer than three reactions.

Resolution of the rate curves for the other soils in Table III yielded results analogous to those described above for Waukegan silt loam, *i.e.*, the observations could be fitted by assuming that there were three simultaneous first-order reactions. In these soils, however, relatively little P was involved in the most rapid reaction. Further experience will be required to determine whether the number of reactions that must be assumed to fit the data is invariably three.

Examination of the P^{32} data indicates that the quantity of P involved in the slow reaction increased linearly with time in contrast to the asymptotic relationship found with the adsorption data. Otherwise, the P^{32} and adsorption data were qualitatively similar, indicating existence of three reactions differing in rate. Except for Waukegan silt loam, however, no detailed analysis was attempted, because the time requirement in centrifuging made the time scale inaccurate. In studying the P^{32}-equilibration curve for Waukegan silt loam, added P^{32} was assumed to equilibrate immediately with 9.1 ppm of soil P^{31} in the solution phase (Table III). The remaining data were corrected for the progress of the slow reaction, using linear extrapolation. It was then found necessary to add 0.75 hour to the time scale before the zero point of 9.1 ppm was brought into line with the remaining points, assuming a reaction first-order with respect to P. Since 0.75 hour was in excess of the time of centrifuging, the results indicate existence of a third reaction, more rapid than the remaining two.

The sources of the P involved in the three hypothetical reactions are unknown. Since several chemical and physical forms may be involved, it is preferable to consider the individual reactions as behavioral patterns characterized by a quantity and a rate rather than some particular chemical species or physical state.

The concept of characterizing the more reactive portion of soil inorganic P as regards both quantity and rate of release appears to be advantageous, since availability of soil P to plants is no doubt likewise a function of both quantity and rate of release. The fact that the techniques described above permit a characterization of soil P by two or three quantities and rates of release may have the additional advantage of adding a degree of flexibility to accommodate

the behavior of different kinds of plants and the effects of environmental conditions. If the rate of P-adsorption by a root is low, and if the absorbing surface remains in one place for a long time, the quantities of P released from the soil by the rapid, intermediate, and slow reactions are probably all of significance in determining the P-availability, but the differences in rates of release between reactions may have little bearing on the result. On the other hand, if the rates of P-absorption and of movement of the absorbing surface through the soil are high, unit quantity of P in rapidly-released form will be of greater availability than unit quantity of P in slowly-released form, because the plant will absorb a greater proportion of the P in the former. How long a root absorbs P at a particular location is not known. For annual plants, however, it is probably not longer than 2 or 3 days. By that time, the original absorbing surface has become inactive, and growth has developed a new absorbing surface at another location.

In the soil, of course, the process by which P arrives at the absorbing surface involves diffusion from the site of release as well as release *per se*. The resin-adsorption technique provides an estimate of the release rate but not the diffusion rate. By mixing the resin with moist soil and allowing the mixture to stand for different lengths of time, however, measurements involving both release rate and diffusion rate may be obtained.

In addition to its use in evaluating P-availability in untreated soils, the resin-adsorption technique provides a means of following the course of fixation of fertilizer P and of evaluating residual effects of previously-applied phosphate fertilizers. The value of the technique for these applications will be enhanced by experimental work to determine the significance to plants of the various quantities and rates for the adsorption reactions under different conditions.

Resin-adsorbed phosphorus and "A" values

A preliminary investigation was made of the suitability of the quantity of P adsorbed by an exchange resin as an estimate of P-availability in soils. Samples of 16 soils were employed, for which estimates of P availability to plants by the method of Fried and Dean[4]) were available. All samples were from the north-central states, and formed a part of the 1951 cooperative series

of experiments described by Dean*). The samples analyzed were those sent to Ames by the original investigators, whereas the "A" values were determined on similar samples sent to Beltsville. Only those samples were analyzed for which the P extracted by the Bray and Kurtz[2]) 0.025*N* HCl — 0.03*N* NH_4F method agreed closely for the samples sent to the two locations.

P adsorbed by Dowex-2 was determined as described under "Methods," using a 2-hour period of contact. In this length of time, the most rapid reaction is completed, the intermediate reaction is nearly completed, and the slow reaction has not progressed far enough to have much effect on the results.

TABLE IV

Phosphorus available to plants by isotope-dilution method, and phosphorus adsorbed by resin and extracted by NH_4F-HCl solution from 16 soils

U.S.D.A. soil number	Soil type	State	Soil phosphorus, ppm		
			Adsorbed by resin	Extracted by NH_4F–HCl solution *)	Available to plants ("A" value)**)
51631	Edgeley silt loam	North Dakota	15.3	6.0	42.4
51629	Fargo clay	North Dakota	28.3	13.5	66.6
51630	Langdon loam	North Dakota	14.1	3.25	27.4
51568	Cheyenne fine sandy loam	North Dakota	5.9	2.5	30.6
51619	Barnes silt loam	Minnesota	17.1	9.0	55.1
51617	Fargo silty clay loam . .	Minnesota	10.3	6.0	30.1
51616	Waukegan silt loam. . .	Minnesota	46.5	28.0	177.1
51618	"Marshall-like" silt loam	Minnesota	8.6	1.75	25.5
51626	Barnes loam	South Dakota	26.0	10.0	69.9
51587	Hastings silt loam . . .	Nebraska	12.8	11.5	48.5
51588	Carrington clay loam . .	Nebraska	3.8	1.5	16.0
51570	Holdrege silt loam . . .	Nebraska	22.7	16.5	62.9
51628	Miami loam	Michigan	8.6	1.75	21.8
51622	Muscatine silt loam . . .	Illinois	5.3	3.0	21.8
51623	Elliott silt loam	Illinois	4.7	1.75	17.1
51585	Munjor silty clay loam. .	Kansas	28.9	22.0	74.6

*) Determined by H. Heidel.
**) Calculated on weight basis.

The data are shown in Table IV. The correlation coefficients were found to be 0.91 for "A" values and P extracted by the Bray and Kurtz[2]) method, and 0.95 for "A" values and P

*) Dean, L. A., Uniform phosphorus experiments: cooperative studies on the fertility status of soils. (Mimeographed.) Unpublished progress report issued by the Division of Soil Management and Irrigation, B.P.I.S.A.E., U. S. Department of Agriculture, Beltsville, Maryland, 1952.

adsorbed by Dowex-2 resin. These results indicate that adsorption of P from soil by an anion exchange resin has some merit as an estimate of soil-P availability. Further work with soils of more widely-differing characteristics will be required to properly validate the theoretical advantages of the resin method.

SUMMARY

Adsorption of phosphate by the anion-exchange resin Dowex-2 was investigated. The resin adsorbed small quantities of P from solution quantitatively. The rate of P-adsorption by resin agitated in solution was proportional to the P-concentration in solution, and was independent of the rate of diffusion of adsorbed P in the resin. When 1 g of soil was shaken continuously with 1 g of resin in 100 ml of water, the rate of P-adsorption by the resin was controlled by the rate of P-release from the soil. Quantities of P adsorbed from soil by resin after different lengths of time were less than those equilibrated with P^{32} during the same time intervals. The curves showing quantity of P adsorbed vs. time could be satisfactorily described by the hypothesis that there were three simultaneous reactions differing in rate, each reaction being first-order with respect to P. The same was true of the P^{32}-equilibration data, except that the rate of the slowest reaction was apparently independent of time. In a group of 16 soils, the correlation between P adsorbed by the resin in 2 hours and P-availability to plants in the greenhouse, measured by the isotope-dilution method of Fried and Dean, was 0.95. The corresponding correlation between P extracted by the 0.25*N* HCl — 0.03*N* NH_4F extractant of Bray and Kurtz was 0.91.

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